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### Genetic Screening of Familial Hypercholesterolaemia.

Cardiovascular pathologies, such as myocardial infarction, cerebrovascular accidents, and peripheral vascular ischemia, are commonly observed in individuals with elevated levels of cholesterol, triglycerides, and low-density lipoprotein (LDL) levels, as these factors are atherogenic. Beyond diet and lifestyle, genetic defects involving lipoprotein synthesis, transportation, and lipid metabolism also contribute to elevated lipid levels. Familial hypercholesterolemia (FH) is one such genetic disorder, inherited in an autosomal dominant manner, leading to the elevation of low-density lipoprotein cholesterol (LDL-C). FH has a global prevalence of 0.32%, affecting approximately 1 in 313 individuals [1]. A recent study by Chua et al., [2] reported a higher prevalence of FH in Malaysia with 1 in 100 individuals affected by FH [2]. Using the Dutch Lipid Clinic Network Score (DLCNS) to screen 5,130 participants, Chua et al., identified 55 potential FH cases, although they reported a low detection rate of less than 1% [2]. Improved detection rates could be achieved with mass parallel molecular identification for mutations in apolipoprotein genes, complemented by family cascade screening. Screening within families where the molecular defect is known is relatively straightforward; however, the numerous mutations associated with FH complicate the genetic analysis, leaving most patients with FH to remain undiagnosed and miss out on potentially life-saving treatments.

The LDL receptor (LDLR) gene is the most common mutation in FH, accounting for approximately 90% of cases, and it plays a key role in the cellular uptake of LDL-C. The next common mutation seen in apolipoprotein B (ApoB), accounts for 5-10% of cases, while proprotein convertase subtilisin / kexin-type 9

(PCSK9) mutations occur in fewer than 3% of cases [3].

In Malaysia, genotyping of clinical FH patients was carried out by Lye S-H et al, [3] and Razman AZ et al, [4]. Lye S-H et al. used a high throughput microarray platform, identifying significant risk-associated single nucleotide polymorphisms (SNPs) in 76.60% of patients. Similarly, Razman AZ et al, used next-generation sequencing (NGS), and identified 41 pathogenic variants across LDLR, ApoB, PCSK9, and LDLRAP1 genes in clinical FH subjects. They concluded that genetically confirmed FH prevalence was approximately 1:427, with a detection rate of 0.2% [4].

#### Implications of Genetic Screening in FH:

Chua et al. estimated that there are about 320,000 individuals with FH in Malaysia. In a primary care or physician's clinic setting, applying the DLCNS screening tool may be time-intensive. However, creating referral pathways for patients with high lipid levels, obesity, diabetes, or a family history of premature coronary events could help streamline the DLCNS process. Those classified as definite, probable, or possible FH could then undergo molecular testing for FH-related genes.

Identifying pathogenic variants in FH remains challenging, especially given the wide range of genes analysed in parallel through NGS. The distinction between mutations (permanent nucleotide changes) and polymorphisms (variants with >1% frequency) often leads to confusion regarding pathogenicity. To address this, the American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommend using "variant" with specific classifications: pathogenic, likely pathogenic, uncertain

significance, likely benign, or benign. Determining variant pathogenicity, especially for newly identified variants, remains complex.

Genetic screening aims to identify individuals with pathogenic variants for timely intervention, typically through the administration of statins or, in the case of PCSK9 mutations, PCSK9 inhibitors. Screening young individuals (under age 45) with acute coronary syndrome could be

especially beneficial, as they may gain substantial advantages from the interventions. These include cholesterol-lowering medications, natural supplements, dietary cholesterol reduction, and lifestyle modifications for overall cardiovascular health.

**Keywords:** *Familial hypercholesterolemia, genetic screening, next-generation sequencing.*

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REVIEW ARTICLE

**Brain-derived Neurotrophic Factor (BDNF) Responses to Exercise in Individuals with Spinal Cord Injury: A Systematic Review.**

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**Abstract**

**Introduction:** Spinal Cord Injury (SCI) leads to debilitating effects. This study aimed to conduct a systematic review examining the correlation between exercise, physical activity, and levels of Brain-Derived Neurotrophic Factor (BDNF) in individuals with SCI. **Method:** A search was conducted between 2013 and 2023 using the PubMed, CENTRAL, Medline, and Wiley Online Library databases, along with bibliographic searching. The evaluation process was conducted at each stage to identify eligible studies. Methodological quality assessments were performed on each eligible study, and the data were presented in tabular form. **Results:** Out of 2,928 papers, only five met the eligibility criteria for inclusion in the review. The methodological quality of the included studies ranged from low to moderate. The demographic variables of the participants, including age, years since injury, gender, level and severity of injury, sample size, study design, intervention, outcome measures, and findings, were organized into a table. All the included studies exhibited heterogeneity. **Conclusion:** The findings suggest a potential association between exercise and physical activity and BDNF concentration levels in individuals with SCI. However, further research with larger sample sizes and rigorous methodology is necessary to establish the long-term effects of exercise and physical activity on BDNF concentration levels. Clinically, this review underscores the importance of tailored exercise interventions in enhancing neuroplasticity and recovery in SCI patients.

**Keywords:** *Brain-Derived Neurotrophic Factor (BDNF), cord Injury, exercise, physical activity, spinal.*



## Introduction

Spinal Cord Injury (SCI) presents significant challenges, leading to permanent disabilities like paralysis and loss of sensation [1]. These injuries not only cause physical impairments but also lead to complex physiological changes, such as autonomic dysregulation, which further complicates the overall health and well-being of individuals with SCI [2]. The annual incidence of SCI is estimated to be between 250,000 and 500,000 cases, with a prevalence of 40 to 80 cases per million individuals [3]. Despite advances in medical research and rehabilitation, individuals with SCI continue to face substantial functional limitations that hinder their ability to perform activities of daily living (ADLs) such as self-care, feeding, and mobility [4]. These impairments also restrict participation in employment [5], sports [6], and social activities [7, 8], often resulting in a diminished quality of life [9].

Exercise and physical activity are known to mitigate some of these impairments, improving muscle strength [10], balance [11], and overall physical fitness [5], which in turn can enhance participation and quality of life [18]. However, the impact of exercise on neuroplasticity and neuronal regeneration in SCI remains underexplored. One key factor in this area is Brain-Derived Neurotrophic Factor (BDNF), which plays a crucial role in the survival, excitability, and regeneration of neurons [6]. BDNF is particularly important in the context of SCI, where neurological impairment and autonomic dysregulation may alter the body's typical physiological responses, including the production of BDNF.

Given BDNF's role in supporting neuroplasticity, its potential to enhance locomotor function through exercise and physical activity makes it a promising target for rehabilitation in SCI patients. BDNF is known to influence brain structures, including the hippocampus and prefrontal cortex, which are involved in synaptic neurotransmission and neuronal proliferation [19]. Although the benefits of BDNF are well-documented in healthy individuals [24], the elderly [25], and other clinical populations such as stroke [27] and

multiple sclerosis patients [28], the responses of BDNF to exercise in individuals with SCI are less consistent and robust. This inconsistency may be due to several factors specific to the SCI population, including the extent of neurological impairment, the level and completeness of the injury, and the adapted nature of exercise modalities used.

Research on the effects of exercise and physical activity on BDNF levels in SCI individuals is scarce, leaving a significant gap in our understanding of how these interventions might influence neuroplasticity and neuronal recovery in this population [3]. In light of these gaps in knowledge, this review aims to provide a thorough analysis of current scientific literature to clarify the potential benefits and mechanisms associated with exercise and physical activity in enhancing BDNF-mediated neuroplasticity and neuronal reconstruction in individuals with SCI. Our hypothesis is that the intensity and duration of exercise may differentially regulate BDNF levels, with more intense or longer-duration activities potentially leading to more pronounced neuroplastic effects.

## Materials and Method

### Literature search strategy

To comprehensively identify relevant studies, a systematic literature search was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The search covered studies published between January 1993 and January 2023. This search was executed across key health-related databases, including PubMed, CENTRAL, Medline, and the Wiley Online Library. Search terms were selected from three categories: Population (Spinal Cord Injury OR Paraplegia OR Tetraplegia), Intervention (Exercise OR Physical Activity), and Outcome (Brain-Derived Neurotrophic Factor OR Neurotrophic). These terms were combined using Boolean operators (AND, OR) to refine the search results effectively. The search was



restricted to studies published in English, which could introduce a language bias, and this should be considered a potential limitation. A secondary search was also conducted through the reference lists of relevant studies to identify additional potential sources. Table 1 below outlines the study search strategy.

### **Inclusion and Exclusion Criteria**

The inclusion criteria were meticulously defined to ensure the relevance and quality of the studies included in this systematic review. Eligible studies met the following criteria: participants were aged 18 years or older, had chronic or subacute SCI, the study was conducted on human subjects, and full articles were available in English. Additionally, studies were required to be published in academic journals between 1993 and 2023. Studies that were systematic reviews, scoping reviews, and narrative reviews were excluded from the analysis. Furthermore, studies were excluded if their outcomes did not include an assessment of BDNF concentration.

### **The Review and Data Extraction Process**

The review process involved three phases to ensure the reliability and validity of the findings. Initially, the main author screened the titles of retrieved papers to identify potentially relevant articles, eliminating irrelevant or duplicate studies. Abstracts of remaining articles were then screened against the inclusion and exclusion criteria. Discrepancies were resolved through discussion and consensus among the reviewers, with a third reviewer consulted if needed. Full texts of eligible studies were retrieved and assessed by the main author. Each article was critically evaluated by two reviewers to ensure alignment with the research question, extract relevant data, and determine the quality of the studies. The data extraction process involved creating a structured table that included information on participants (age, gender, years since injury), study characteristics (design, sample size, intervention, outcome measures),

and outcomes (BDNF concentration changes, exercise effects).

### **Methodological Quality Assessment**

The methodological quality of each study was assessed using the Joanna Briggs Institute (JBI) checklist, which is designed to evaluate a wide range of study types, including experimental, observational, and cross-sectional studies. The JBI checklist is comprehensive, covering key aspects such as sample selection, study design, data collection methods, statistical analysis, and the appropriateness of conclusions. Its use ensured a consistent and rigorous assessment of methodological quality across studies, systematically identifying potential biases and limitations. The checklist's flexibility and comprehensiveness enhanced the transparency and robustness of the review, making it a suitable tool for evaluating the quality of included studies. The JBI instrument exhibits notable face validity and acceptance, demonstrating its suitability for research and clinical settings. Moreover, it is straightforward to administer and does not require much evaluation time. The JBI's evaluation criteria include:

- A representative sample
- An appropriate apparatus
- An adequate sample size
- An appropriate description of study reports, subjects, and setting;
- Adequate data coverage
- Validity of condition measurement
- Reliability of condition measurement
- Appropriate statistical analysis
- The adequacy of the response rate

The evaluation of eligible research was conducted by two reviewers, with any discrepancies resolved through consensus discussion or by consulting a third reviewer.

## Results

### Study search results

The search yielded 2,928 studies: 29 from PubMed, 1,560 from Medline, 16 from CENTRAL, 1,322 from Wiley Online Library, and 1 from a bibliographic search. After eliminating 328 duplicates, 2,600 studies remained for review. The titles of 2,549 studies were evaluated for eligibility, resulting in 127 potentially eligible studies. The abstracts of the 127 studies were then reviewed according to the inclusion criteria, and 122 studies were excluded, leaving five articles that met the inclusion criteria. These five studies were subsequently analyzed, and the necessary data were extracted. Figure 1 illustrates the flowchart of the search strategy and selection process, adhering to PRISMA guidelines.

### Study Methodological Quality

Overall, the studies demonstrated moderate methodological quality. Many studies had low methodological quality, primarily due to small sample sizes, which increased the risk of bias and exaggerated results [1-6]. Most studies employed suitable sampling techniques when selecting research participants [1, 3-6]; however, one study raised concerns about the reliability of its sampling procedures [2]. None of the included studies met the criteria for an acceptable sample size [1-6]. Additionally, the eligible studies did not provide an accurate representation of the participants or the specific environments in which the studies took place [1-6]. The condition identification methods utilized in all studies were deemed valid, with consistent and dependable measurements across participants in many investigations [1-6]. All studies employed appropriate statistical analyses [1-6]. Moreover, all eligible studies had sufficient response rates, and instances of low response rates were adequately addressed [1-6]. Please refer to Table 2 for the quality assessment of all included studies.

### Characteristics of the Participants

Participants' average age ranged from 35 to 56 years [1-6]. The years since injury (YSI) among participants ranged from eight to 31 years [1-6]; however, two studies did not report YSI [3, 5]. Male participants predominated in these studies [1-3], though two studies did not specify the number of male and female participants [5, 6]. One study reported an equal number of male and female participants [4]. Most participants were tetraplegic rather than paraplegic [1-3, 6]. Two studies included seven participants with complete injuries [4], while two studies reported 19 participants with incomplete injuries [1, 2, 4]. The remaining two studies did not specify the number of participants based on injury level [5, 6]. Table 3 displays the characteristics of the participants.

### Characteristics of the Studies

The six included papers used various study designs, including prospective observational [1], repeated measure [2], cross-sectional [3], and prospective cohort designs [4], while two studies did not specify their design [5, 6]. All studies had small sample sizes, ranging from 10 to 16 participants, with a total of 74 participants across all studies [1, 2, 4, 6]. The types of exercise and physical activity varied, including wheelchair marathons [1], wheelchair rugby training [4], gait training on a treadmill [3], single-bout gait training, graded exercise gait training using a treadmill with body weight support (BWST) [2, 5], and hand bike incremental exercise [6]. The summary of the study characteristics is tabulated in Table 4.

### The Relationship of Exercise and Physical Activity on The Level of BDNF Concentration

As previously indicated, the exercises and physical activities used in the included studies varied. Two studies reported significant immediate effects on BDNF levels after a 1-hour half marathon ( $p < 0.05$ ) and 10 minutes of hand bike incremental exercise (60 rpm, 20W/5 minutes) [1-3]. Tetraplegic participants showed

higher BDNF levels ( $p = 0.0055$ ) compared to paraplegic participants ( $p = 0.0312$ ) after the 1-hour half marathon [1]. Two studies reported no significant changes in BDNF levels following exercise: one after BWST gait training and a treadmill run ( $p > 0.05$ ) [4], and another after wheelchair rugby training at rest, during training, and cooldown ( $p > 0.05$ ) (5). One study found that BDNF levels were nearly regulated after high-intensity exercise (HIE) compared to moderate and low-intensity activities ( $p = 0.05$ ) [3].

## Discussion

### Quality of the included studies

This review examines the correlation between exercise, physical activity, and BDNF levels in individuals with SCI. Despite an exhaustive search of studies published between 1993 and 2023, only six studies met the criteria for inclusion [29-34]. This scarcity highlights a significant gap in research on how exercise and physical activity influence BDNF levels in this population. The moderate methodological quality of these studies points to several challenges specific to SCI research, such as variability in study design, demographic characteristics, injury severity and levels, functional limitations, health considerations, and rehabilitation intervention which contribute to methodological issues.

Key limitations observed in the included studies encompass the type of study design, small sample sizes, recruitment challenges, and the complexity of exercise interventions. These constraints may have affected the reliability, introduced bias, and limited the generalizability of the reported findings. To enhance future research, it is imperative to prioritize rigorous study designs such as randomized controlled trials (RCTs) and prospective cohort studies [43]. These designs offer better control over confounding variables and enable the identification of causal relationships between exercise, physical activity, and BDNF levels.

The heterogeneity among the included studies further complicates the synthesis of results and

the formulation of robust conclusions. Variations in study designs, exercise interventions, and outcome measures contribute to inconsistent findings, making it challenging to establish clear patterns or causal relationships. Exercise modalities, intensity levels, and outcome measures differed across studies, leading to diverse and sometimes conflicting results. The decision to include a range of study designs and interventions was made to provide a comprehensive overview of existing evidence on BDNF responses to exercise in the SCI population. However, this diversity also highlights the need for standardized protocols in future research. Standardization would improve the consistency and comparability of findings, facilitating more accurate assessments of the impact of different exercise interventions on BDNF levels.

Personalized exercise programs are crucial given the variability in BDNF responses to different interventions. Future research should aim to develop and implement standardized protocols for exercise interventions and outcome assessments. Additionally, a thorough examination of participant characteristics, including the extent, intensity, and duration of SCI, as well as demographic factors such as age, gender, and length of injury, would provide a more nuanced understanding of BDNF responses. Such data would enable more precise subgroup analyses and enhance the contextual understanding of results.

Most studies on BDNF have been conducted on animals, likely due to ethical and safety considerations in human research [44]. While animal studies have shown positive effects of exercise on BDNF levels, these findings may not always translate reliably to humans [45]. Therefore, emphasizing human studies with larger sample sizes is essential for making reliable recommendations regarding exercise and physical activity for individuals with SCI. The findings from this review underscore the need for more rigorous, standardized research to better understand the relationship between exercise,

physical activity, and BDNF levels in the SCI population, ultimately informing more effective rehabilitation strategies.

### **The Influence of SCI Individual Characteristics on the level of BDNF**

Several factors could have impacted the BDNF responses to exercise in the included studies. One significant factor is age. Age-related changes in neurobiology, such as decreased neuroplasticity and altered BDNF regulation [46, 47], can influence how individuals respond to exercise. Older adults may experience different BDNF responses compared to younger individuals, potentially affecting the generalizability of findings across different age groups.

Gender is another critical factor [47]. Research suggests that gender differences may affect BDNF levels and neuroplasticity. Hormonal variations and sex-specific biological mechanisms might lead to differential responses to exercise between males and females. The studies reviewed often had limited gender diversity, which could impact the overall findings and their applicability to both sexes.

Injury severity also plays a crucial role in determining BDNF responses [48]. Individuals with more severe injuries may experience greater challenges in neuroplasticity and functional recovery. Variations in injury severity among study participants could contribute to inconsistencies in BDNF levels and their response to exercise interventions. A more granular analysis of injury severity in future research could provide insights into how different levels of injury affect BDNF responses.

Time since injury is another important factor [48]. The stage of injury recovery can influence BDNF levels and the effectiveness of exercise interventions. Early post-injury periods may see different BDNF dynamics compared to later stages of recovery. Thus, including a range of time since injury in studies is crucial for understanding how BDNF responses to exercise evolve over time.

### **The Influence of Exercise and Physical Activity on BDNF Levels in Individuals with SCI**

The review highlights the complex and varied relationship between exercise, physical activity, and BDNF levels in individuals with SCI. BDNF is a crucial protein involved in neuroplasticity, neuronal survival, and cognitive function, making it a key target in SCI rehabilitation [50, 51]. BDNF is a key player in neuroplasticity and functional recovery after SCI [49, 51]. It promotes neuronal survival, growth, and differentiation, which are essential for repairing and regenerating damaged neural circuits [49, 51, 52]. Exercise and physical activity have been shown to elevate BDNF levels, potentially enhancing neuroplasticity and aiding functional recovery [50, 52].

Current understanding indicates that BDNF contributes to functional recovery by supporting the growth of new neuronal connections and improving synaptic plasticity [49, 51, 52]. In the context of SCI, elevated BDNF levels may facilitate the reorganization of neural pathways and enhance motor and sensory function [49, 52]. However, the precise mechanisms through which exercise-induced BDNF increases translate into functional improvements remain an area of ongoing research.

However, the evidence regarding the optimal exercise types and intensities for enhancing BDNF levels in this population remains inconclusive. One of the most significant findings from this review is the variability in BDNF responses to different exercise types and intensities among individuals with SCI. High-intensity exercises, such as half-marathons, graded intensity protocols, and High-Intensity Interval Training (HIIT), have shown more promise in increasing BDNF levels than low-intensity or short-duration exercises [29-34]. These high-intensity activities seem to create a more substantial physiological stimulus, which may be necessary for the upregulation of BDNF production in the central nervous system [52]. This observation suggests that exercise intensity

plays a critical role in modulating BDNF levels and highlights the limitations of a one-size-fits-all approach to exercise prescription in SCI rehabilitation.

The variability in BDNF responses can be attributed to several factors, including the type of exercise, its intensity, and the duration of the intervention. For example, endurance activities like wheelchair marathons [34] or graded exercise [30] protocols appear to encourage greater BDNF production compared to lower-intensity exercises. The mechanistic basis for this may lie in the physiological demands these exercises place on the body, leading to increased neurotrophic support [49, 51]. However, the review also points out that not all individuals with SCI may be capable of participating in high-intensity exercises due to their physical limitations, health status, or injury severity. Therefore, while high-intensity exercises may be beneficial for some, they may not be suitable for all, emphasizing the need for personalized rehabilitation programs.

In addition to intensity, the duration and consistency of exercise play crucial roles in influencing BDNF levels. The review indicates that short-term or sporadic exercise interventions may not be sufficient to sustain elevated BDNF levels over time. None of the included studies demonstrated that exercise and physical activity could stimulate sustained BDNF production over prolonged periods [29-34]. This finding suggests that while exercise can acutely boost BDNF levels, maintaining these benefits may require ongoing, long-term engagement in physical activity. Therefore, future research should prioritize long-term interventions with multiple follow-ups to better understand the lasting effects of various exercise modalities on BDNF levels in individuals with SCI.

Moreover, the relationship between exercise, BDNF levels, and broader rehabilitation outcomes remains underexplored. While BDNF is a marker of neuroplasticity [51, 52], its role in functional recovery after SCI is not fully understood. The review suggests that future studies should include broader outcome measures

to evaluate the potential associations between BDNF levels and cognitive function, physical fitness, mobility, and pain. These measures would provide a more comprehensive understanding of how BDNF modulation through exercise influences overall rehabilitation outcomes in SCI patients.

Therefore, the review underscores the importance of exercise intensity in modulating BDNF levels in individuals with SCI but also highlights the variability in responses based on the type and duration of exercise. This variability suggests that a one-size-fits-all approach to exercise prescription may not be effective for BDNF enhancement in SCI patients. Instead, personalized rehabilitation programs should be developed, taking into account the specific type and intensity of exercise most likely to induce BDNF production while also considering the physical capabilities and safety of each individual. High-intensity exercises, particularly those used in HIIT protocols, appear promising for BDNF elevation and could be prioritized in rehabilitation settings. However, further research is needed to confirm these findings and to explore the long-term effects of various exercise modalities on BDNF levels, as well as their broader impact on functional recovery in SCI patients.

## Conclusions

In conclusion, this systematic review highlights the potential of exercise and physical activity to influence BDNF levels in individuals with SCI. Although findings suggest a possible link between specific exercise modalities and increased BDNF concentrations, the evidence remains limited due to moderate level of study quality, small sample sizes and heterogeneity of the included studies. Further research with larger, well-designed studies is needed to establish clear recommendations. Clinically, these findings support the integration of tailored, high-intensity exercise protocols in SCI rehabilitation to potentially enhance neuroplasticity and functional recovery.

### **Clinical implications**

This review reveals that exercise may significantly elevate BDNF levels, enhancing neuroplasticity and neuronal regeneration in individuals with spinal cord injury (SCI) [49, 50, 51, 52]. Incorporating targeted exercises like aerobic training, resistance training, and high-intensity interval training (HIIT) into rehabilitation programs could improve outcomes in motor function, sensory recovery, and overall quality of life for SCI patients. Clinicians can personalize these exercises based on the patient's injury severity, health status, and specific functional goals, maximizing the benefits of rehabilitation. For instance, Paraplegia Fitness Integrated Training (PARAFiT), introduced by Hisham et al. [5], is an example of a regimen that may elevate BDNF levels, although further research is needed to confirm its effectiveness to elevate BDNF level. The variability in BDNF responses across different exercise types emphasizes the importance of individualized rehabilitation strategies. These findings provide a strong foundation for developing more effective, evidence-based rehabilitation protocols to enhance recovery in SCI patients.

### **Limitations of the study**

The main constraint of this analysis is the small number of eligible studies, with only six studies meeting the inclusion criteria. Furthermore, the study also have moderate level of quality and demonstrated significant heterogeneity in terms of study designs, participant characteristics, interventions, and outcome measures. This heterogeneity complicates direct comparisons, impedes the feasibility of a meta-analysis, and potentially weakens the overall conclusions, introduce biases, impacting the accuracy and reliability of the findings.

### **Recommendations for future research**

Future studies should focus on larger, well-designed clinical trials that can more definitively establish the long-term effects of exercise on

BDNF levels and neuroplasticity in the SCI population. Such research will be crucial in refining exercise prescriptions and maximizing the therapeutic benefits of rehabilitation for individuals with SCI. Additionally, exploring the underlying mechanisms by which different exercises influence BDNF production could provide deeper insights into how these activities promote neuroplasticity and neuronal regeneration.

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### **Authors contributions**

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Full manuscript: Hafifi Hisham

Table 1. Search strategies

Criteria	Databases
Databases	PubMed, Medline, CENTRAL, Wiley Online Library
Keywords	‘Spinal Cord Injury OR Paraplegia OR Tetraplegia’ AND ‘Exercise OR Physical Activity’ AND ‘Brain-Derived Neurotrophic Factor OR Neurotrophic’
Limiters	Full Text; English, 1993-2023, academic journals, spinal cord injury population
Search modes	Boolean/Phrase

\*This table shows the search strategies in health-related databases for literature searching

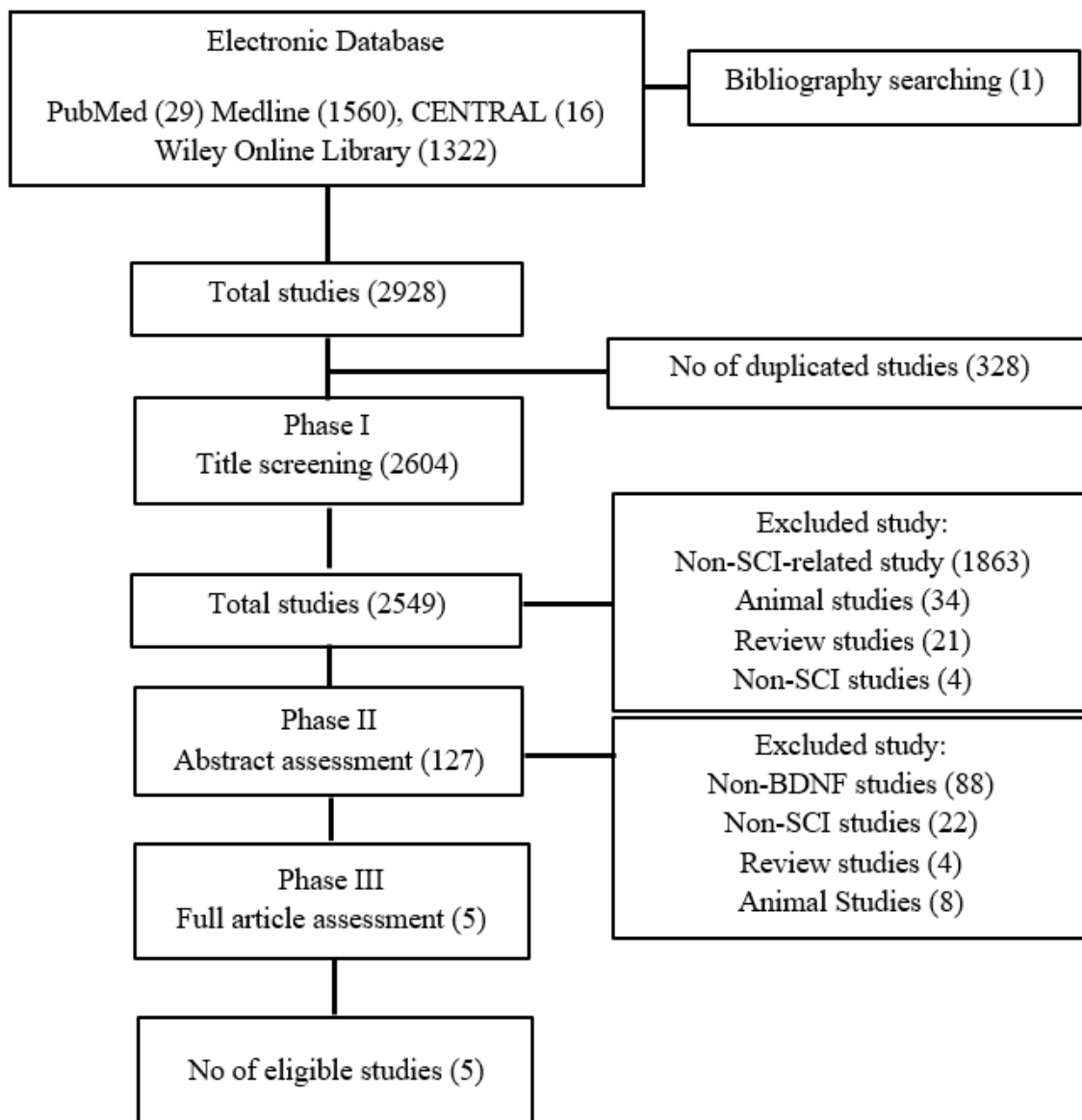


Figure 1. Search results



Table 2. JBI Critical Appraisal Checklist for Studies Reporting Prevalence Data.

No.	Criteria	Studies					
		Nishimura et. al (2022)	Goldhardt et al. (2019)	Leech and Hornby, (2017)	Zeller et al (2015)	Harness et al (2014)	Vega et. al (2008)
1.	Was the sample frame appropriate to address the target population?	Yes	Yes	Yes	Yes	Yes	Yes
2.	Were study participants sampled appropriately?	Yes	Yes	Unclear	Yes	Unclear	Unclear
3.	Was the sample size adequate?	No	No	No	No	No	No
4.	Were the study subjects and the setting described in detail?	Yes	No	No	Yes	No	No
5.	Was the data analysis conducted with sufficient coverage of the identified sample?	Yes	Yes	Yes	Yes	Yes	Yes
6.	Were valid methods used for the identification of the condition?	Yes	Yes	Yes	Yes	Yes	Yes
7.	Was the condition measured in a standard, reliable way for all participants?	Yes	Yes	Yes	Yes	Yes	Yes
8.	Was there an appropriate statistical analysis?	Yes	Yes	Yes	Yes	Yes	Yes
9.	Was the response rate adequate, and if not, was the low response rate managed appropriately?	Yes	Yes	Yes	Yes	Yes	Yes

Table 3. The participants' characteristics

Author	Sample size (n)	Age Mean (SD)	YSI Mean (SD)	Gender	Level of SCI	Severity of injury
Nishimura et. al (2022)	16	Para: 56 (4) y/o Tetra: 35 (4) y/o	Para: 381 (63) months (31 years)  Tetra: 161 (35) months (13 years)		Para (n): 8 Tetra (n): 9	
Goldhardt et al. (2019)	10	40.6 (9.03) y/o	3.5 (1.27) years	Male (n): 5 Female (n): 5	C4 – C7 (n): 3 T1 - L5 (n): 7	Complete (n): 2 Incomplete (n): 8
Leech and Hornby, (2017)	11	41 (14) y/o	103 (85) months (8.5 years)	Male (n): 8 Female (n): 2	C4-C7 (n): 10 T4 (n): 1	Chronic incomplete (n): 11
Zeller et al (2015)	11	31.7 (5.9) y/o		Male (n): 11	C5-C7 (n): 11	Complete and incomplete
Harness et al (2014)	15	31.8 (10.9) y/o	63.9 (54.4) months	Male (n): 12 Female (n): 3	C4-C7 (n): 10 T2-L1 (n): 5	Complete (n): 5 Incomplete (n): 10
Vega et. al (2008)	11	40.6 (6.3) y/o			Para (n): 11	ASIA A and B

\*\*YSI: Years Since Injury, y/o: Years old, Para: Paraplegia, Tetra: Tetraplegia, C: Cervical, T: Thoracic, L: Lumbar, ASIA: American Spinal Injury Association. This table shows the demographic data of the included participants.

Table 4. Characteristics of the included studies.

Author	Study design	Type of exercise and PA	Main result	Conclusion
Nishimura et. al (2022)	Prospective observational study	Half marathon wheelchair race and physical training before the race	Pre-race: No significant difference in BDNF level between the LSCI and CSCI After the race (1 hour): A significant difference between CSCI (P = 0.0055) and LSCI (P = 0.0312) After the injury: Returned to the baseline level (1 hour)	BDNF in LSCI and CSCI was increased immediately after the race
Goldhardt et al. (2019)	Cross-sectional (crossover design)	A single session of gait training with a treadmill and seven days later with a walker	No significant differences during pre- to post-intervention for TS and WS (p>0.05): TS, Pre: 434.02 (184.02) pg/mL Post: 341.31 (152.35) pg/mL WS, Pre: 261.22 (182.21) pg/mL Post: 277.96 (130.05) pg/mL	A single bout of gait training with a BWS treadmill or walker without BWS is not able to alter BDNF levels.
Leech and Hornby (2017)		Graded-intensity body weight supported treadmill	The BDNF was nearly increased with HIE (p = 0.05) whereas no significant increase in the low intensity of the exercise group (p = 0.56).	Serum BDNF concentrations were modulated by the exercise intensities
Zeller et al (2015)	A prospective cohort	Warm-up (10 min): Continuous pushing, 20-meter submaximal sprints (8 times), agility drills. UL stretching The main training (45 min): Ball handling, passing drills, scrimmage activity, tactical practice, game simulation Cool down: Moderate continuous pushing	No significant differences in BDNF concentration level during (p >0.05): At rest: 33.2 (21.6) ngml <sup>-1</sup> After warming up: 31.9 (18.9) ngml <sup>-1</sup> After training: 29.9 (11) ngml <sup>-1</sup>	A typical wheelchair rugby training session does not affect basal serum BDNF concentration in elite SCI athletes
Harness et al (2014)		30 minutes of LB, BWS TT, WBV	No effect of exercise on BDNF, (p >0.05): F(4, 52) = 0.14, P = 0.97, with baseline levels of BDNF at 2.37 (1.41) ngml <sup>-1</sup>	Acute changes in BDNF were not observed.
Vega et. al (2008)		Hand bike incremental exercise test until exhaustion (increased to 20 W every 5 minutes for 60 r.p.m	BDNF increase after 10 minutes of exercise at approximately 1.5-fold from basal level (P<0.05).	short moderate intensity hand biking increases the BDNF level immediately but in long-term effects.

\*\*CSCI: Cervical Spinal Cord Injury, LSCI: Lumbar Spinal Cord Injury, HIE: High Intensities Exercises, OM: Outcome Measure, BWS: Body Weight Supported, BWS TT: Body Weight Supported Treadmill Training, TS: Treadmill Session, WS: Walker Session, WBV: Whole Body Vibration, FES: Functional Electrical Stimulation (FES), LB: Load Bearing, UL: Upper limb, ELISA: Enzyme-linked immunosorbent assay, W: Watts, pg/ml: Picogram/Mililiter, ngml<sup>-1</sup>: Nanogram per Milliliter. This table shows the search strategies in health-related databases for literature searching.

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## REVIEW ARTICLE

# A Review of the Experience of Clinical Attachment among Undergraduate Nursing Students and Clinical Instructors.

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### Abstract

**Introduction:** Clinical attachments benefit nursing students but present with challenges. This 2018-2023 review looks at the experience of instructors and students with clinical attachments to identify areas for development. **Aims:** This literature review aims to look for literature related to the experience of nursing students and clinical instructors during clinical attachment as well as how the ratio of student-to-clinical instructor affects both parties. **Methods:** The studies included in this study were identified through two databases: Google Scholar and ScienceDirect with keywords such as “*experience*”, “*clinical attachment*”, “*clinical instructors*”, “*challenges*”, “*effect*”, “*inadequate*”, “*insufficient*”, “*ratio*”, and “*nursing students*”. The published studies from 2018 onwards were included. **Results:** A total of 14 articles were analysed in this review after screening over 2639 articles available online. It was found that students were tasked with workload, unclear goals, and negative staff interactions. Instructors struggle to balance student needs, self-care, and workload. High student-to-teacher ratios affect both groups. **Conclusion:** Understanding these experiences improves clinical learning and prepares future nurses for practice.

**Keywords:** *Clinical instructor, experience of clinical attachment, nursing students, review.*

## Introduction

Clinical placements, which serve as a fundamental aspect of nursing education, provide a platform for the application of theoretical knowledge in the context of actual patient care. These attachments offer invaluable opportunities for students to refine their skills, cultivate professionalism, and enhance their confidence as aspiring healthcare providers. However, guaranteeing high-quality clinical experiences requires going beyond superficial observations and exploring the first-hand experiences of the individuals directly engaged in the process: the students and the clinical instructors.

This review seeks to accomplish this objective by examining the existing body of literature on clinical attachment experiences within the past five years (2018-2023). Through the synthesis of important discoveries, we aim to provide insight into the positive experiences, difficulties, and areas for enhancement in this essential component of nursing education.

Multiple recent studies emphasise the crucial significance of clinical learning environments in moulding student growth. The study conducted by Wan Mamat et al. (2023) highlights the difficulties encountered by nursing students in Malaysia, such as heavy workload, unfavourable staff attitudes, and ambiguity in learning goals[1]. From the instructor's point of view, research investigates the intricacies of their role in overseeing and directing students. Dağ et al. (2019) identified challenges such as effectively managing workload, balancing the diverse learning needs of students, and ensuring self-care to prevent burnout[2]. The study conducted by Nuryani et al. (2022) underscores the importance of efficient communication and collaboration between educators and learners to enhance the supervisory connection[3].

In addition to personal obstacles, the study conducted by Altundal et al. (2022) highlights the influence of student-to-instructor ratios on the calibre of clinical encounters[4]. Instructors face difficulties in meeting student expectations and delivering personalised feedback when dealing

with high ratios, which may impede the development of skills and confidence.

This review examines various relevant studies to gain insight into the common and unique experiences of nursing students and clinical instructors. Our goal is to enhance the clinical learning environment for future nurses by identifying their strengths, weaknesses, and areas for improvement. This will help them better prepare for the challenges of real-world healthcare practice.

## Research objectives

Therefore, in this literature review, the authors aimed to review the available studies that demonstrated the experience of nursing students and clinical instructors during clinical attachment as well as how the ratio of student-to-clinical instructor affects both parties.

## Methodology

In the literature review search process, two databases were used: ScienceDirect and Google Scholar. The authors curated pertinent research published within the last five years (2018-2023) for inclusion in this study, guided by key terms such as “*experience*”, “*clinical attachment*”, “*clinical instructors*”, “*challenges*”, “*effect*”, “*inadequate*”, “*insufficient*”, “*ratio*”, and “*nursing students*”.

Initially, 2639 articles surfaced based on the keywords mentioned above. Subsequently, 2146 were excluded due to duplication, or because they fell under exclusion criteria, such as not being published within the last 5 years, or not aligning within the field of study. An additional 22 were also excluded as it is not an academic journal. On the final note, 14 articles were included and analysed in this review. The process of reviewing the literature was conducted systematically through the application of the PRISMA flow diagram, as depicted in figure 1. The full texts of all articles considered for inclusion were acquired and thoroughly examined independently.

## **Results**

### ***The experiences of clinical instructors in managing students during clinical placement***

From 14 studies, eight (8) studies state the experience of clinical instructors in managing students during clinical placement. These studies were conducted in Turkey, Indonesia, Iran, Egypt, and the United States. The integration of clinical placement is imperative for nursing students, as it furnishes them with pragmatic encounters and the opportunity to familiarise themselves with authentic clinical environments, which is indispensable for their instruction as healthcare practitioners. The efficacy of clinical education largely hinges on the pivotal role of clinical mentors, who offer guidance and oversight to students during their placement. This specific subtopic endeavours to scrutinise and appraise the difficulties confronted by clinical mentors in effectively overseeing students throughout their clinical placements.

The management of nursing students by clinical instructors during clinical supervision is a multifaceted and intricate experience, involving both gratifying and demanding elements. Clinical instructors have documented different encounters during clinical guidance, encompassing obstacles linked to the scarcity of resources from both the tertiary educational establishment and the clinical milieu, which influenced their guidance experience. Moreover, it is recommended to establish a welcoming clinical learning environment by fostering efficient communication among the stakeholders to enhance the clinical guidance experience [5]. The experiences of clinical instructors during clinical supervision encompass the task of instructing students in the practical application of theoretical knowledge, adjusting their responsibilities to cater to the specific needs of students, and valuing the chance to gain knowledge from students. Ensuring effective clinical learning environments necessitates providing support and resources to clinical supervisors [6].

Numerous predicaments arise when it comes to managing students. The predicaments that impact clinical supervision in nursing education, as identified by Amin et al. (2022), encompass non-constructive learning environments characterised by student overcrowding, limited apparatus, adverse attitudes in the clinical milieu, physician-oriented education, and the inefficiency of the education-treatment system. Clinical mentors encounter difficulties in addressing limitations within the clinical milieu, which might culminate in the exclusion of formal clinical education. A comprehensive scrutiny of the actual experiences of nursing mentors revealed that the successful accomplishment of nursing education objectives necessitates the presence of proficient mentors capable of overseeing students in clinical educational environments. Nevertheless, if the mentors are predominantly focused on rectifying the limitations in the clinical context, they will not reap any noteworthy advantages other than marginalising formal clinical teaching [7]. According to the investigation by Amin et al. (2022), participants frequently observed nurses and supervisors concealing apparatus to hinder its utilisation, which had a detrimental effect on the work milieu. Clinical instructors face challenges in managing their clinical workload while overseeing nursing students. Establishing effective communication and collaboration with students, considering their unique learning requirements, can prove to be challenging. The scarcity of resources and time constraints present significant hurdles in providing comprehensive supervision and feedback. The presence of conflicts and divergent expectations between students and instructors gives rise to obstacles in the supervisory relationship. Addressing the diverse learning backgrounds and experiences of pupils poses a complexity. Keeping up with healthcare innovations and integrating evidence-based procedures into supervision presents a demanding task. Supervising in various healthcare environments poses challenges. Ensuring adherence to professional boundaries and ethical considerations presents a formidable



challenge [2]. Striking a balance between workload and practising self-care to avoid burnout presents a formidable challenge for educators [2]. Nurse educators encounter challenges in effectively balancing their clinical responsibilities with supervising nursing students. Nursing educators frequently face obstacles when it comes to effectively communicating and collaborating with nursing students. This is due to the need to consider the distinct learning needs and preferences of these students. Additionally, they may encounter difficulties in providing comprehensive supervision and feedback to nursing students due to limited time and resources. Conflicts or differences in expectations between nursing students and clinical instructors can create challenges in the supervisory relationship, leading to difficulty in maintaining professional boundaries and adhering to ethical norms [3,8,9]. Nurse educators face challenges in adapting to the varied learning backgrounds and experiences of nursing students. Furthermore, nurse educators may encounter challenges in staying updated with healthcare advancements and incorporating evidence-based practices into clinical supervision. Moreover, they may face additional complexities while delivering supervision in diverse clinical settings and scenarios. Nurse educators frequently face challenges in efficiently managing their workload and practising self-care to mitigate burnout and sustain their effectiveness [3,8,9].

In research conducted by Ahmari Tehran et al. (2021), the emerging field of ineffective educational training emphasised several concerns, one of which was the lack of a student numerical scheme. A participant conveyed that collaborating with a significant multitude of learners and coordinating internships for the entire week will inevitably lead to exhaustion for both educators and students, consequently diminishing the standards of education. Clinical instructors face difficulties in fulfilling the distinctive needs and obligations of learners as certain individuals expect diverse assignments

and responsibilities rather than repetitive and prolonged workshops [10].

### ***The impact of the student-to-clinical instructor ratios on clinical teaching experiences***

From 14 studies, seven (7) studies state the impact of the students-to-clinical instructors ratios on clinical teaching experiences. These studies were conducted in Turkey, Iraq, Iran, Malaysia, Philippines, and Oman. The quality of clinical teaching experiences holds significant importance in the preparation of future healthcare professionals. Nevertheless, a notable challenge in healthcare education is the considerable student-to-clinical instructor ratio. This ratio can have adverse effects on both students and clinical instructors in terms of their ability to provide effective supervision.

In a study conducted in Iraq by Attia and Ibrahim (2023), it was highlighted that inadequate clinical instructors may encounter difficulties in effectively guiding and mentoring students in clinical settings, thereby impacting the quality of nursing education. These instructors may struggle to offer constructive feedback to students, thereby impeding their learning and personal development. The insufficiency of clinical instructors due to the high number of students can hinder the development of competencies and skills among nursing students as they may not receive effective guidance and evaluation based on competency indicators [12]. This issue can create challenges in assessing the level of student competencies, and clinical instructors may be unable to provide the necessary support and mentorship to nursing students, thereby affecting their learning experience and overall preparedness for clinical practice. Ultimately, inadequate clinical instructors can have a detrimental effect on students' competencies, evaluation processes, and overall learning experience in clinical settings [12]. A study conducted in Turkey by Altundal et al. (2022) revealed that nursing students have certain expectations from instructors in clinical practice,

such as the provision of explanations using appropriate examples and adopting a fair approach[4]. However, due to the increasing issue of an imbalanced ratio, clinical instructors find it challenging to meet these expectations as they have other wards and students to attend to, with limited time available for each ward visit. When clinical instructors are overwhelmed by the imbalance between students and themselves, the effectiveness of clinical teaching and learning experiences for nursing students may decline. Clinical instructors play a crucial role in supervising students' clinical activities and empowering them to acquire clinical competency and skills [13]. Insufficient clinical instructors may lead to a higher student-to-clinical instructor ratio, which in turn limits individualised attention and feedback for students. Due to a scarcity of clinical instructors and their limited availability and accessibility, the potential for timely feedback and constructive evaluation processes may be impeded. Moreover, the insufficiency of clinical instructors may also have an adverse impact on their role, thereby potentially affecting both student learning and patient safety [13]. According to a study conducted by Padagas (2020), the constraints of time and resources may hinder clinical instructors from offering adequate supervision and guidance to nursing students, consequently hindering the students' learning experience. This issue is often observed when clinical instructors are unable to attend to all the students in a single ward and are required to oversee multiple wards. Additionally, a dearth of support and guidance from clinical instructors can result in a decrease in students' confidence and competence [15]. Inadequate supervision and monitoring by clinical instructors can act as a deterrent to effective clinical education [10]. This can be attributed to the high student-to-clinical instructor ratio, which necessitates catering to a large number of students within the limited presence of clinical instructors.

## Discussion

This study scrutinised the experiences of nursing students and clinical instructors during their clinical placements. Through an extensive exploration of relevant literature and the synthesis of significant discoveries, it has provided illumination on a range of obstacles and prospects for augmenting this pivotal facet of nursing education.

Clinical instructors encounter a multitude of difficulties in their supervisory position, encompassing the need to address constraints within the clinical environment, effectively manage their workload, and navigate conflicts and divergent expectations between students and instructors. These challenges can impede the provision of comprehensive guidance and feedback to students, thereby influencing their learning experience and level of preparedness for clinical practice. Furthermore, the scarcity of resources, time limitations, and the necessity to strike a balance between clinical responsibilities and self-care present formidable obstacles for clinical educators. To add on, a study from Ugwu et al. (2023) stated some students experiencing mixed experiences during clinical attachment: negative and positive feelings, with some voicing out regarding poor clinical supervision and lack of equipment[16]. Boman et al. (2022) says that nursing students encountered a state of perplexity regarding their sense of self and resorted to employing distinct tactics to manage insufficiencies within their educational surroundings[17]. The introduction of an internship program for nursing students in their final year was discovered to improve clinical aptitude, boost self-assurance, and equip students for enhanced professional proficiency [18].

A deficiency in clinical educators could significantly impede the educational experience of nursing learners. The reduced availability of educators may lead to insufficient personalised attention for students, thereby affecting their skill enhancement and academic advancement. Furthermore, prompt feedback and evaluations

might be postponed, resulting in a stressful educational setting [19]. The nonexistence of clinical educators could also restrict student independence, thereby jeopardising their capacity to cultivate crucial competencies.

The insufficiency of clinical instructors exacerbates the obstacles encountered by nursing students, such as emotional turmoil, susceptibility, and challenges in translating theoretical knowledge into practical application. In the absence of seasoned mentors, students might encounter difficulties with ambiguity, seclusion, and navigating intricate clinical scenarios [20, 21, 22]. Additionally, the absence of instructors could escalate emotional reactions like apprehension and unease, thus impeding students' coping mechanisms with the exigencies of clinical placements. Effective guidance by experienced nurses, pivotal for fostering student assurance, is compromised in such circumstances [22].

Moreover, a deficit in clinical instructors may fuel negative learning atmospheres, heightened moral distress, and jeopardise students' professional ethos [23]. Without ample assistance, students might grapple with exasperation, remorse, and a weakened sense of direction. These adversities underscore the critical necessity for supplementary resources and assistance to ensure the welfare and triumphant progress of nursing students.

The discussion in the paper also addresses the crucial factor of how the student-to-clinical instructor ratios impact the experiences of clinical teaching. When clinical instructors are not enough, there might be an uneven distribution of students and instructors, leading to a shortage of personalised attention and feedback for the students. This imbalance has the potential to impede the development of nursing students' competencies and skills, as well as hinder the timely provision of feedback and constructive evaluation processes. Ultimately, this circumstance may jeopardise the calibre of clinical education and the welfare of patients.

Several studies referenced in the discourse emphasise the adverse consequences of elevated student-to-clinical instructor ratios within the realm of nursing education. Matters such as fatigue experienced by both instructors and students, difficulties in meeting the expectations of students, and insufficient supervision resulting in diminished self-assurance and proficiency among students are discussed [4, 10, 11, 12, 13, 14, 15]. Additionally, the limitations imposed by time and resources worsen the obstacles encountered by clinical instructors in delivering efficient supervision and guidance to nursing students.

## **Conclusion**

Clinical instructors play a pivotal role in shaping the prospective nurses of tomorrow. However, their experiences shed light on a multitude of difficulties pertaining to the management of students. These difficulties however are not restricted to requirements in assets, enormous student-to-clinical instructor proportions, and changing instructive necessities. Both instructors and students are affected by these obstacles, impeding the effectiveness of supervision, feedback, and ultimately, the outcomes of learning.

The field of nursing education is confronted with notable challenges resulting from a critical deficit in clinical educators. The scarcity of proficient faculty members hinders students' development of essential clinical competencies, timely feedback reception, and the fostering of indispensable professional assurance. Consequently, this scarcity yields detrimental impacts on student welfare, moral advancement, and overall academic accomplishment. To address these challenges, it is imperative to make significant investments in augmenting the pool of clinical educators and furnishing them with necessary resources. Moreover, it is essential to explore innovative approaches, such as peer mentoring and simulation-based teaching, to support student understanding and alleviate the

consequences of faculty deficiencies. By giving precedence to establishing a robust framework for clinical education, nursing programs can elevate the preparedness of prospective nurses to adeptly navigate the intricate demands of the healthcare environment.

Numerous avenues exist to enhance the experiences of clinical attachments. Optimising the allocation of resources, fostering collaboration between educational institutions and clinical sites, and exploring alternative teaching methodologies such as simulation laboratories could help alleviate the burden on instructors. It is essential to tackle the issue of uneven student-to-instructor ratios to offer customised attention and feedback. Moreover, mentorship programs and workshops could equip instructors with the essential skills to manage various learning styles and integrate evidence-based practices.

By acknowledging these challenges and actively seeking out solutions, all parties involved can cultivate a supportive and enriching environment for clinical learning, benefiting both students and instructors. Consequently, this will ensure that future nurses graduate with the confidence, competence, and critical thinking abilities essential for thriving in the realm of healthcare.

### **Conflict of interest**

The author declared there is no conflict of interest in this study.

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### **Ethical approval**

Ethical approval for this study has been obtained from IREC (IIUM Research Ethics Committee).

### **Authors contributions**

In this study, Muhammad Afiq Ikhmal and Nursyafiah Yasmin were responsible for reviewing the related paper. Muhammad Afiq Ikhmal, Nur Ain, and Noor Maizatul Akma designed the methodology, and Muhammad Afiq Ikhmal wrote the manuscript. Nur Ain was responsible for counter checking and reviewing the related academic paper utilised, the methodology implemented and proofread the manuscript.

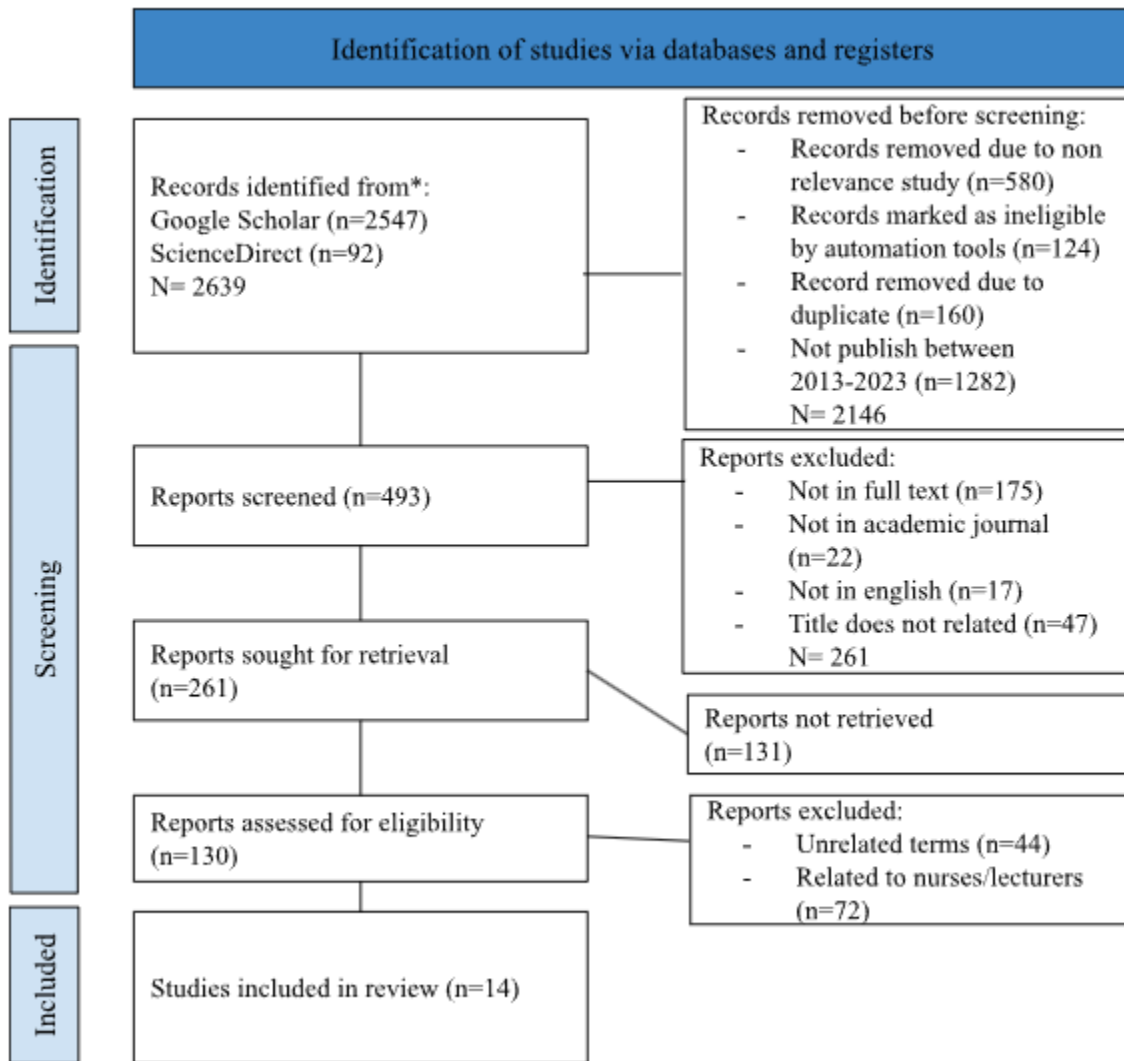


Figure 1. PRISMA flowchart on the experience of clinical attachment

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## REVIEW ARTICLE

### Pathogenic Advances in Rheumatoid Arthritis: A Review.

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#### Abstract

Rheumatoid arthritis (RA) affects approximately one percent of people worldwide. It falls within the category of an inflammatory immune-mediated illness where the primary tissue involved is the joint. Environmental and genetic factors combine to cause RA. However, the exact onset of the disease is unknown, as does the appropriate time to diagnose it as RA. In RA, the synovial membrane and surrounding tissues are attacked by the immune system. The pathophysiology of RA still raises three unanswered problems. First, how the environment or heredity drives the immune system. Secondly, how it persisted in causing inflammation in the surrounding joint, and thirdly, how inflammation results in damage to bones. There are various numbers of cells associated with the progression of RA disease. Proinflammatory cytokines are mostly produced by macrophages, which can also serve as antigen-presenting cells. In joints, the fibroblast-like synoviocytes (FLS) interact with cells of the innate host immune system and activate B and T cells causing an increase in chemokines and cytokines production such as TNF- $\alpha$ , IL-1, and IL-6 thus allowing a feedback loop to occur. B cells, T cells, and macrophages will all play a part in these extra encounters. Matrix metalloproteinases (MMPs), prostaglandins (PGs), and inflammatory cytokines are also produced in varying quantities by the activated fibroblast-like synoviocytes within the synovial membrane. The accumulation of chemokines and nitric oxide (NO) also contributes to inflammation and tissue catabolism. MMPs enter the synovial fibroblast (SF) directly as a result of the positive feedback loop, which can lead to the degeneration of bone and cartilage. We herein summarized the key pathogenic advances addressing these issues, along with the basics of rheumatoid arthritis, its mediators, and the cell signalling pathways involved.

**Keywords:** *Inflammation, mediators, rheumatoid arthritis.*

## Introduction

The term Rheumatoid Arthritis (RA) is based on the Greek words, which means *watery* and *inflamed* joints [1]. RA disease is categorized as an immune-mediated inflammatory disease in which the joint is the main site of tissue. The inflammation within the joint causes redness, warmth, swelling, and pain. It has been identified as one of the primary causes of disability [2], a reduction in the expectation and quality of a person's life, the possibility that a patient may become clinically impaired within 20 years [3], the increased risk of cardiovascular disease, early mortality, infection, and cancer, and the potential to cause death [4]. Rheumatoid arthritis (RA) affects approximately range from 0.24 to 1%, but varies considerably around the world [5].

As it is described as the most typical disease of systemic autoimmune connective tissue, common features include symmetric joint inflammation, and continuous degradation of articular cartilage and periarticular tissues [6]. It manifests as inflammation of the synovial membrane around diarthrodial joints, synovial vaginae, and gliding synovial bursae, which causes localised destruction [7]. This disease is a chronic type involving certain organs and occurs by systemic effect throughout the body [1].

RA is a combination of genetic and environmental factors [5]. RA is commonly identified by its symmetrical pattern. One of the most distinguishing features from other inflammatory arthritis is relying on its ability to expand the disease to new joints [8]. The central new criteria are clinical synovitis, involving the number of affected joints, abnormality in serology, increased acute phase, and duration of symptoms [9].

## History and Epidemiology

Around 1500 BC, Ebers Papyrus uncovered an equal case matching rheumatoid arthritis. Subsequently, multiple research findings indicate that mothers from diverse generations exhibit physical abnormalities that are recognised as pathognomonic for arthritis [10].

RA involves 1% of the world's population, and in the United States only, more than 30 billion dollars has been put into managing the prevalence of this disease [11]. The prevalence studies are unrecorded from evolving areas of countries. It has been reported in annual published reports recently that in Northern European and North American nations, RA may differ between 20 and 50 cases per 100,000 and probably lessened in Southern European areas [9].

The Arthritis Foundation of Malaysia estimates that 5 out of every 1000 individuals in the nation have RA, with women accounting for 75% of the disease's victims [12]. As it affects 1% of people around the world, with the highest frequency is in women between 40 and 60 years of age [7]. Based on gender, women are 2:1 to 3:1 strongly affected by this disease compared to men, with the yearly occurrence of RA stated to be around 40/100,000 globally [10].

The highest range age for RA onset is between 45 and 65 years [4] which is commonly the time of hormonal transition in women. Some ethnic groups have a higher possibility of being infected based on the distribution differences and interactions of genetics and environment [13]. RA is available in all regions of the world where it has been investigated. It is assigned as the universal type of disease, where there is no information on any areas or ethnic groups which does not involved with this disease. The challenges of the descriptive epidemiology of RA are based on chronic and complex diseases, and it is unclear how early we should be calling it RA [9,10].

Life expectations of RA are declining about 3 to 10 years compared to the general population, fatality rates are also greater among RA sufferers, and this has not been transformed over the last 2 to 3 decades. The major factor of death in RA patients is based on complications of pulmonary, various secondary infections, hematological diseases, and cardiovascular and gastrointestinal problems [9,13].

## **Aetiology of RA**

Hormonal, genetic, immunological, reactive oxygen species, infectious agents, physiological, and environmental factors interact to cause the complex disease known as RA. Dietary factors components, pollutants, and urbanization also may be the other factors involved [6].

Like many other diseases, RA is a result of a combination of environmental and hereditary factors. 50% to 60% of the development factors for RA are inherited. Genes critical to immune responses are encoded by the major histocompatibility complex (MHC); the MHC allele linked with these genes was first identified as HLA-DR4. Previous research demonstrated the relationship between RA [14] and HLA-DR4, HLA-DRB, and a number of additional alleles classified as common epitopes. PTPN22 is the non-MHC gene in RA. It carries impulses through the T cell receptor and influences lymphocyte development. Genome-wide association studies have discovered over 100 additional loci that impact susceptibility to RA. A few are mentioned in Table 2.1. These genes related to their cellular function that control immune reactions which supports the concept of autoimmune disease in RA.

The investigation into the involvement of epigenetics in RA is still in its early stages. Their methods are crucial for understanding how environmental cues affect gene expression. The complexity of RA analysis is made possible by the apparent existence of epigenetic modifications in certain cells, such as lymphocytes or synovial fibroblasts [15].

One of the extrinsic risk parts of RA development and severity is smoking. Smoking triggers the citrullination process by modifying the HLA-DR-restricted immune activity into autoantigens, this is the procedure that turns arginine amino acid into citrulline. The development of autoimmune diseases is triggered by autoantibodies directed against citrullinated proteins [16]. Previous research has also suggested that smoking may cause the lungs' enzyme that is in charge of citrullination to express itself, hence triggering

the autoantibody's antigen targets [17]. Infection by several bacterial and viral pathogens has been considered a cause of RA, although direct infection of RA joints has not been reported [18]. The study of microbiome is a recent part of RA study and another autoimmune disease. This complex task involved viewing the various microbiomes that exist on the skin, gastrointestinal, and respiratory tracts [19]. Other variables that may also raise the risk of RA include birthweight, breastfeeding, birthplace, and socioeconomic status [10].

## **Pathogenesis of RA**

Rheumatoid arthritis will induce immune system to target synovial membrane and tissues near joints. Three unanswered problems remain regarding the pathophysiology of RA. First, how the systemic immune response is triggered by the environment or genetics. The second is how the inflammation persisted in the surrounding joint, and the third is how the inflammation causes damage to the bone [20].

The first stage involves the hallmark of autoimmune priming in the appearance of Rheumatoid factors (RFs) and/or the anti-citrullinated protein antibody (ACPA) which is categorized as the RA-associated autoantibodies. At this stage, the increased serum biomarkers of inflammation and pro-inflammatory cytokines can also be discovered. RFs are autoantibodies targeting the antigen through recognizing a domain of the IgG Fc portion, further, they form immune complexes that contribute to the disease process. The appearance of RF positively correlates with the severity of disease, while the ACPA identifies proteins which undergone the conversion of arginine to citrulline and predicts the severity of disease such as the degree of joint destruction, thus making ACPA much more specific for RA than RF [21].

The next phase of RA is the emergence of clinical arthritis based on the joint inflammation level that is adequate to produce clinical signs and symptoms. The triggers including trauma and infection start to localize in the systemic

autoimmune action to the joint [15]. Activated cells of the innate system recognize the unknown triggers or microbial pathogens, which is done mainly by antigen-presenting cells (APC) like macrophages and dendritic cells via intracellular pattern recognition receptors (PRRs). The molecules of the innate system including APC and effector cells are recruited locally and move to nearby lymphoid tissues when they are unable to defeat the pathogen alone [22]. From there, the APC cells manifest arthritis-associated antigens including the activated B cells to T cells [23]. This start inflammation in the joint and aligns the disease with the local mesenchymal cells, which are synovial fibroblasts (FLS) [24]. The proliferation of synovial fibroblasts and extensive inflammatory cell infiltration such as CD4<sup>+</sup> T-cells and cells of the innate immune system cause the hyperplasia of the synovial membrane [20]. The complex interactions in RA synovium between various cellular constituents resulting the production of various cytokines and other inflammatory mediators. The cytokines released will induce blood vessels in membrane of synovia to divide in a process called hypervascularization. The increased blood flow allows the excess synovium tissue growth and leads to the thickening of the synovium [15].

The third phase of RA is inflammation involving the destruction of cartilages, bones, tendons and ligaments. To support synovial expansion and inflammation, angiogenesis occurs to provide spaces for inflammatory cells and their sustained nutrients. The thick synovial membrane, called pannus occupies the small space between the joint's bones and it covers the bone's surface and the articular cartilage. The cytokines initiate the differentiation of monocyte precursors into osteoclasts leading to bone erosion [15]. However, there are still unclear distinctions regarding the underlying mechanisms between these three stages. This is based on the damage to the joint that can be detected in the very early clinical diagnosis of RA by sensitive imaging techniques [25].

There are various numbers of cells involved in the development of RA. The macrophages are the basic element of proinflammatory cytokines and may also perform as antigen-presenting cells [26]. The CD4<sup>+</sup>T-cells, specifically IL-17-producing helper T (Th17) cells contribute to the enrolment of RA systemic immune response. The Th17 cells' migration into the inflamed site is assisted by mesenchymal cells to produce homeostatic proliferation and further stimulate IL-17 production. In joints, the fibroblast-like synoviocytes (FLS) interact with cells like macrophages, dendritic cells, mast cells, and NK cells, as well as cells of the adaptive immune system, B and T lymphocytes [9]. The activation of B and T cells caused an increase in chemokines and cytokines production such as TNF- $\alpha$ , IL-1, and IL-6 thus allowing a feedback loop to occur. This will involve the additional interactions of B cells, T cells, and macrophages. The activated fibroblast-like synoviocytes also produced various amounts of matrix metalloproteinases (MMPs), prostaglandins (PGs), and inflammatory cytokines within the synovial membrane [23]. The accumulation of chemokines and nitric oxide (NO) also contributes to inflammation and tissue catabolism [27]. The positive feedback loop causes the direct invasion of MMPs into the synovial fibroblast (SF) and can cause cartilage and bone destruction [23]. The hallmark cytokine of the recently identified "Th17" T helper cell population, IL-17 (also called IL-17A), has been linked to the development of a number of autoimmune disorders, including rheumatoid arthritis. A novel category of cytokines with strong pro-inflammatory characteristics was founded by IL-17. Research on mice, mammalian cell culture methods, and clinical settings all point to IL-17's potential role in aggravating rheumatoid arthritis [23-25].

#### **a) Synovial fibroblast**

The two visible layers of normal synovial tissue are the subintima/sublining layer, which is mostly made up of two cell types, and the surface layer, also known as the intima/lining layer or synovial

lining. The cells include type A macrophage-like synoviocytes (MLS) and type B fibroblast-like synovial cells (FLS). The FLS is the most commonly present in the rheumatoid joint [28]. It generates the extracellular matrix (ECM) components of the synovial fluid that is essential for lubrication of the joint cartilage integrity. It detains the mononuclear cells in synovium and allows neutrophils to accumulate in the joint space, further regulating the leukocyte aggregation of two areas to eliminate debris from the synovial cavity. The SF also excrete various connective tissue types, including fibronectin and collagen [28].

There are review evidences that relates FLS as major cause of rheumatoid arthritis. The synovial hyperplasia marks the RA which the subintima layer amplify into 15 or more cells thus increases the FLS number. Three different mechanisms involved in this process; that are the hyperproliferation of FLS, reduced apoptosis and senescence.

The FLS secretes inflammatory mediators including IL- 1, 4, 6, 8, 10, 12, 13, 17, 18, 21, TNF- $\alpha$ , TGF- $\beta$ , IFN- $\gamma$ , VIP, iNOS, and via upregulation of cyclooxygenase-2, prostaglandin E2 during inflammatory response. The resting T cells have been presented to activate the FLS. The direct contact between RA FLS and T cells through the connection of T Cell CD47 receptors on the surface of synovial fibroblast leads to T cell activation, T cell cytokines production and T cell proliferation. RA-SF will induce growth of blood vessels by producing proangiogenic factors, including fibroblast growth factors (FGF), vascular endothelial growth factors (VEGF) and IL-18 in order to sustain pannus formation in arthritis process [28]. FLS shows the number of signal transduction pathways activation specifically of inflammatory responses. Nuclear factor- $\kappa$ B (NF- $\kappa$ B) is known as a key transcription factor in RA. The activation of NF- $\kappa$ B regulates the matrix metalloproteinases (MMPs) expression showing it may regulate the

joint destruction based on in vitro. While *in vivo* study, NF- $\kappa$ B expression is elevated at joint destruction sites which specifically at the cartilage-pannus junction [23].

#### **b) Macrophages**

In RA, synovial macrophages and monocytes involvement in driving the pathways are still an issue [30]. The matured monocytes enter the bloodstream undergo trans-endothelial migration into synovia. Both cells play their roles in the inflammatory initiation and perpetuation, the migration and adhesion of leukocytes, matrix degradation, and angiogenesis [31]. During joint inflammation, they become activated and control the pro-inflammatory cytokines and enzyme production [32] together with infiltrating macrophages/monocytes thus leading to cartilage and bone destruction [33].

Various cell-surface receptors are expressed by macrophages in chronic RA such as chemokine receptors and cell adhesion molecules (CAMs). Chemokine receptors on macrophages engaged in the aggregation of monocytes/macrophages to sites of inflammation. CAMs control macrophages interaction with other cells. Integrins such as VLA-4 and VLA-5 also facilitate the attachment of monocytes to endothelium during the transmigration process into the synovium. The integrin-dependent activation of macrophages leads to the initiation of transcription factor pathways and inflammatory mediator production including matrix metalloproteinases (MMPs) and cytokines [31].

Interleukin-1 and TNF- $\alpha$  are the pro-inflammatory cytokines released by RA synovial-tissue macrophages and exert overlapping effects in RA. TNF- $\alpha$  activates interleukin-6 production, which is formed by synovial-tissue macrophages and synovial-fluid monocytes. Synovitis primarily is cytokine driven. The initial key observation was when the rheumatoid synovial cell cocultures decreased IL-1 production when infused with neutralizing anti-TNF antibodies resulting with. Another study also shows that the

other pro-inflammatory cytokines including IL-6, IL-8, and GM-CSF were also induced by TNF in cocultures of RA synovial cells. The observations lead to the understanding of the macrophage-produced TNF- $\alpha$  concept as the key mediator of disease and driving the other proinflammatory cytokines among the other cytokines [30].

### c) T cells and B cells

Most RA patients carry the cluster of HLA-DRB1 epitope. On the HLA-DR  $\beta$ -chain, these genotypes have a similar amino acid sequence that, through interaction with certain peptides, influences how antigen is presented to T-cell receptors (TCRs). The arthritis-related peptides may exist in disease-associated HLA-DR alleles. It caused the stimulation and augmentation of autoantigen-specific T cells in the joints and lymph nodes [23].

T cells in RA joint commonly have a memory CD4<sup>+</sup> phenotype, express numerous activation markers, and often exist close to antigen-presenting cells (APCs) such as activated B cells, macrophages, and dendritic cells [26]. It stimulates macrophages and synovial fibroblast production and induces rheumatoid synovitis [34]. B cells generate Ig and autoantibodies such as rheumatoid factor and anti-collagen antibodies which build immune complexes that can induce local inflammation [26]. B cells are also presented as antigen-presenting cells (APCs) that can activate the pathogenic T cells [35].

In RA, the tissue-localized B cells reveal their pathogenic properties by definite mechanisms including the production of autoantibodies, T-cell activation, and cytokine synthesis [35]. In immunologic action, T helper cells (Th cells) aid the B cell's maturation into memory B cells and plasma cells and also assist the activation of cytotoxic T cells and macrophages. It divides immediately and secrete cytokines which regulate the active immune response. These cells can differentiate into one of several subtypes including Th1 and Th2 [36]. In normal environments, Th1 and Th2 cells are involved in autoimmunity and respectively mediate immune

reactions against intracellular and extracellular pathogens [37]. In the immune response towards self-antigens, the naïve T cells including Th1 and Th2 were transformed into CD4<sup>+</sup> and CD8<sup>+</sup> effector cells subsets [38]. Both CD4<sup>+</sup> and CD8<sup>+</sup> T cells are the main producers of TNF- $\alpha$  [38]. TNF- $\alpha$  induces IL-1 and IL-6 secretion thus indirectly influencing the Th17 population [38], which is the main key player in autoimmunity. Th17 usually reacted in immune responses against extracellular fungi and bacteria [37]. Pathogenic Th17 cells are the main players in disease development by mediating the growth of pannus, osteoclast genesis, and synovial neoangiogenesis [37]. IL-6 also can trigger *de novo* differentiation of naïve T cells to Th17. The Th17 cells and Th17-cell-derived cytokines further encourage the B-cells proliferation, differentiation, class-switch recombination, and the production of antibodies. This process proved the presence of a positive feedback loop in the inflammatory reaction between B and T cells [35].

### d) Neutrophils

An essential component of the innate immune system are neutrophils. They rectify the infection through the phagocytosis process and by the release of neutrophil extracellular traps (NETs) [39]. Around 60% of neutrophils are present from all types of leucocytes in blood circulation and are one of the earliest cells to reach the synovium during RA [40].

In RA patients, these infiltrated neutrophils are being degranulated which is different from healthy neutrophils. They are also available in synovial fluid and synovial membranes for a few days instead of hours. This apoptosis delay is caused by the existence of anti-apoptotic cytokines such as TNF, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-8, and hypoxia, further increasing the neutrophil survival for up to several days in the affected joints and leads to progressive tissue damage due to their extended life span [41]. The defects in apoptotic neutrophil clearance also can produce

autoantibody production by expressing their autoantigens on their surface [41].

The synovial fibroblast-neutrophils can trigger the proliferation of T-cell stimulation [26] and shift via chemotactic gradients approaching the pathogens before finally binding through the pattern recognition receptors or opsonic receptors [41]. Phagocytosis occurs when the phagocytic vesicle of neutrophils encloses the pathogen and discharges the contents of cytoplasmic granules into the vesicle [42]. During phagocytosis, the plasma-membrane-bound NADPH oxidase is triggered and releases oxygen free radicals and reactive oxygen species (ROS) [43].

The inflammatory neutrophils in RA interact with other cells and release cytokines and chemokines such as the secretion of MMP-8, MMP-9, the cytokines repertoire (IL-1 $\beta$ , IL-6, IL-12, IL-17, IL-18, IL-23, and TNF- $\alpha$ ) and also chemokines (CCL-2, CCL-4, CCL-5, and CXCL-8) [44]. The cytokines derived by neutrophils further regulate the responses with immune complexes, and the soluble immune complexes trigger higher ROS secretion and also granule enzyme [41]. The tissue damage occurs if large numbers of neutrophils infiltrate the tissues and produce large amounts of cytotoxic products that could decrease the antioxidant and anti-protease protective systems in tissues [42].

### **Effector Molecules and Pathogenic Mediators of RA**

The evidence from previous study specify that the vessel dilate as the blood flow to the injured harmed region may increase up to ten-fold. This is mainly caused by the mediators including nitric oxide, prostaglandins (PGE<sub>2</sub>) and inflammatory cytokines. The mechanisms by which bone erosion occurs in RA are not clear [25].

#### **a) Nitric Oxide**

The short-lived signalling molecule, Nitric oxide (NO) is a chemical modulator of inflammation. It has a main role in various physiologic activities such as blood vessel tone, functions of mitochondrial, inflammation, and apoptosis [45].

The synoviocytes, chondrocytes, and endothelial cells are the major producers of NO in inflammatory joints. It is synthesized through the oxidation of L-arginine by nitric oxide synthases (NOS) [46]. The isoforms of NO are expressed as calcium-dependent (cNOS and eNOS) and calcium-independent inducible enzymes (iNOS). cNOS produces the NO and controls the physiological function, while iNOS produces excessive amounts of NO and allows inflammatory disease pathogenesis [47].

NO is associated with RA pathophysiology through inflammation and the destruction of autoimmune-mediated tissue, thus influencing the catabolism of cartilage. Studies and evidence reported that increased endogenous NO synthesis is responsible for T-cell dysfunction in RA. Under physiological conditions, NO regulates T cell functions through the potential of mitochondrial membrane of T cells and able to induce or inhibit apoptosis. During activation, the antigen-specific T cells interact with antigen-presenting cells and lead to the proliferation and differentiation of CD4 T cells. This formation of immunological synapse produces two main subsets of primary effector cells which are T helper cells 1 (Th 1) and Th 2. They are further specifically characterized by their specific patterns of cytokine expression. The Th1/Th 2 cell balance is essential in chronic inflammatory diseases [45].

The main source of NO in RA is the inflamed joint, which synoviocytic fibroblasts are the main source in rheumatoid synovium. Farrell et al.,[48] reported elevated levels of NO which were measured indirectly as nitrite from synovial fluids and serum in RA patients. Macrophages, neutrophils, endothelial cells, osteoblasts, osteoclasts, and fibroblasts can produce NO in the inflamed synovium [49].

NO may perform as a pro-inflammatory mediator together with other mediators including cytokines such as TNF and IL-1 $\beta$  to generate the production of synovial Matrix metalloproteinases (MMPs)[50]. A close network between iNOS expression and MMP has been suggested by



Yoshida et al.,[51] through the involvement study of NO to the production of MMP-1 in the uterine cervical fibroblast cells. In the cellular reactions to hypoxic and inflammatory conditions, the iNOS and MMP3 expressions controlled by transcription factor Hypoxia-inducible Factor 1-alpha (HIF-1 $\alpha$ ) and thus are involved in the pathogenesis of RA [52]. These suggested the roles of NO as the activator of MMPs, but the mechanisms are still in partially understood.

### **b) Matrix metalloproteinase (MMP)**

In chronic joint inflammation, elevated levels of different inflammatory cytokines trigger the pannus and generate numerous proteolytic enzymes causing joint tissue degradation [53]. There are four classes of proteolytic enzymes in matrix proteins involved in cartilage degradation that are; serine/threonine proteases, cysteine proteases, aspartic proteases, and metalloproteases. Matrix metalloproteinases (MMPs) are one of the key mediators of the cartilage, bone, synovial fluid, and adjacent soft tissue resorption. In normal joints, the synthesis and degradation of matrix proteins are in equilibrium, but in RA patients the serum levels of MMPs are increased. This shows that the matrix MMPs can mediate the occurrence and development of RA [53].

MMPs are generated by synovial lining cells, sub-lining fibroblasts, infiltrating leukocytes and macrophages [54]. The MMPs regulations cultivate at the gene transcription level and by the activation of proenzymes [47] and their gene expression is regulated by TNF-alpha and IL-1 $\beta$  via the signal transduction pathways, such as by mitogen-activated protein kinases (MAPKs). The MAPKs target the AP-1 and Ets sites in the MMP promoters and demanding for expression. Under the influence of c-Jun/c-Jun homodimers or c-Fos/c-Jun heterodimers, the protein complex AP-1 is phosphorylated by c-Jun N-terminal kinase (JNK) to activate a DNA-binding AP-1 complex. Therefore, the AP-1 also activates the c-fos and c-jun mRNAs synthesis in reaction to inflammation stimulates [55].

MMP-1 (collagenase-1) is generated by the synovial cells that line the joints and primarily is the first collagenase that forms when RA disease occurs in the synovial fibroblasts. It is localized on the superficial surface of cartilage and its expression is generally 10-fold superior to MMP-13 expression [55]. MMP3 (stromelysin 1) has been studied as the major enzyme generated by fibroblasts and macrophage-like cells in the synovium among the other MMPs, and the amount is higher in synovial fluids of RA patients [52]. In a variety of cell types basically, MMP-3 expression is induced by IL-1 [56] and further can initiate the activation of other MMPs and degenerate multiple proteins, including fibronectin, cartilage link protein, and collagen types IV, VII, IX, and XI [55].

The cytokine-induced transcription factor, Nuclear factor-kappa B (NF-kB), is a major signaling pathway in controlling MMP gene expression. It controls various inducible inflammatory genes such as TNF-alpha, IL-1 beta, and IL-6 [57]. Inhibiting the NF-kB might be crucial therapeutically. A study was conducted by Campbell et al. [58], where insufficient p50 subunits in the animal models were more affected by collagen-induced arthritis. NF-kB is a dimer with p50 and p65 subunits in the cytoplasm and its activation is acquired for the transcriptional induction of MMP-1, as their promoters contain canonical binding sites for NF-kB [59].

### **c) Prostaglandin E2 and Cyclooxygenase2 (COX2)**

The small potent inflammatory lipid mediators, prostaglandins (PG), have been reported to increase in RA patients' synovial fluid and synovial membrane. They are generated by nearly all nucleated cells from the essential fatty acids (EFAs) and are available in most organs and tissues. Their production is typically low in normal tissues but increases shortly due to acute inflammation from the association to leukocytes, leading to pro-inflammatory and anti-inflammatory reactions [60].

PGE<sub>2</sub>, a product of PGH<sub>2</sub> is the main prostaglandins generated by macrophages, synovial fibroblasts and chondrocytes which induced by trauma and pro-inflammatory cytokines IL-1 $\beta$ , IL-6 and TNF- $\alpha$ . It has inhibitory response on NF- $\kappa$ B through ERK-dependent and independent pathways in RA-synovial fibroblast [60].

Prostaglandins are produced when arachidonic acid (AA) is released from diacylglycerol in the plasma membrane via phospholipase-A<sub>2</sub> (plpA<sub>2</sub>) and is delivered into the cyclooxygenase pathway [61]. In the pathway, the AA is processed by cyclooxygenase (COX), prostaglandin endoperoxide H synthase (PGHS), and prostaglandin synthase enzymes, thus inducing the prostaglandins (PG) production. During the sequence, cyclooxygenase (COX) catalyzed the AA to bioactive prostaglandin (PGH<sub>2</sub>). Next, the PGH<sub>2</sub> is catalyzed by specific PGE synthases (PGES) into bioactive PGs that are PGE<sub>2</sub>, PGF<sub>2</sub> $\alpha$ , PGD<sub>2</sub>, PGI<sub>2</sub> (prostacyclin), and TXA<sub>2</sub> (thromboxane), while PGE synthase (PGES) catalyzed the PGE<sub>2</sub>, it also activates the G-protein-coupled PGE receptors (EP) which are EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub> and EP<sub>4</sub>. In RA, the EP<sub>4</sub> receptor plays a pro-inflammatory role in the disease pathogenesis [62].

Prostaglandin E synthase (PGES) has been classified into 3 forms including microsomal PGES-1 (mPGES-1), microsomal PGES-2 (mPGES-2) which are membrane-bound enzymes; and cytosolic PGES (cPGES). In inactive RA, the mPGES-1 is minimally expressed, and the initiation is controlled under COX-2 expression [60]. COX-2-derived PGE<sub>2</sub> is known to have a role in tissue repair, fever, pain, inflammation, and cancer. In recent studies of gene targets, they found that mPGES-1 served as a novel target for anti-cancer and anti-inflammatory drugs [64]. The mPGES-2 is synthesized as a Golgi membrane-associated protein and associated with PGE<sub>2</sub> generation by binding of COX-1 and COX-2. Cytosolic PGES (cPGES) is not committed in any pro-inflammatory stimuli but it is binding to

COX-1 to stimulate immediate PGE<sub>2</sub> released [60].

The cyclooxygenases catalyzed the first two steps in prostaglandins (PGs) biosynthesis, and have 2 major isoforms including COX-1 and COX-2. They are commonly used and targets for nonsteroidal anti-inflammatory drugs, thus explaining their functions in pain, tumorigenesis, and inflammation [65]. Both isoforms are encrypted by different genes and have definite patterns of expression. COX-1 is regularly expressed in various types of tissues. It is important to perform essential metabolic functions for the survival of PGs. While the expression of COX-2 is induced by stimuli or inflammatory mediators such as mitogens, cytokines, growth factors, bacterial endotoxins, and tumour promoters, further incorporated in the development of cancer and various pathogenesis such as apoptosis, angiogenesis, immune surveillance, cell differentiation, invasion and metastasis [60]. The COX activity inhibition is needed to restrain the PGs synthesis in inflammation cases such as RA [66], but the inhibition of COX-1 and COX-2 production by traditional NSAIDs frequently related to side effects including gastrointestinal bleeding, due to both isozymes suppression [60].

#### **d) The inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ and IL-6)**

The cytokines are synthesized by nearly all cells [67] and promote RA autoimmunity by regulating chronic inflammatory synovitis which leads to adjacent joint tissue destruction. The term 'cytokine' is originally from two Greek words combination that are "cyto" (cell) and "kine" (move) [68]. The categorization of cytokines is not practicable due to the simultaneous pleiotropic effect, but to understand the entire mechanism in RA pathogenesis. Cytokines have four categories; pro-inflammatory, inflammatory cytokines mainly in joints, anti-inflammatory, and natural cytokine antagonists [69].

Increased proinflammatory cytokines are identified in joint fluids and synovium of RA

patients including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin (IL)-1 [8]. By targeting the endothelium, both IL-1 and TNF initiate the inflammatory mediators which act as endothelial adhesion molecules inducers during the emigration process into tissues [67]. They act locally and can affect the system such as being involved in cardiovascular disease and osteoporosis [69], also can cause inflammation, fever, tissue destruction and to be the worst, shock and death [67]. The inflammatory cytokines are mostly the pro-inflammatory cytokines that can be detectable in the joints of RA patients. They include IL-1, TNF-alpha, IL-15, IL-16, IL-17, IL-18, interferon (IFN)- $\gamma$ , and granulocyte macrophage-colony stimulating factor [69]. The anti-inflammatory cytokines such as IL-4, IL-10, IL-13, and transforming growth factor (TGF) suppress or block the intensity of inflammatory cascade by inhibiting the generation of IL-1, TNF, vascular adhesion molecules and also chemokines, including IL-8 [67]. An adequate amount of anti-inflammatory cytokines concentrations causes an imbalance in the local area and inefficiency in mediating the counter-regulatory action across the dominant pro-inflammatory cytokines [69], thus representing the inflammation in RA. The natural cytokines antagonists are known as IL-1 receptor antagonists (IL-1ra), IL-18 binding protein, soluble type 2 IL-1 receptor, and soluble TNF receptor (sTNF-R1). They are involved in the mechanism of self-limiting or self-controlling by the immune system, but their observance levels in fluid and synovial tissues are inadequate and the present knowledge about them is not entirely known [69].

During the pathogenesis of RA, it is cytokines play a main role. Cytokines function in synergy to sustain their generation and increase the inflammatory process. Furthermore, they communicate with each other at the level of production such as IL-17 elevating the TNF and IL-1 production by monocytes. Together TNF, IL-17, and IL-1 trigger stromal cells to generate IL-6, thus these cytokines combination produce

synergistic or additive effects. The synergistic effects between TNF, IL-17, and IL-17 occurred in variety cell types including synoviocytes, chondrocytes, osteoblasts, or myoblasts [72].

#### **e) Tumour Necrosis Factor-alpha (TNF- $\alpha$ )**

TNF released by activated fibroblasts and macrophages induced the release of additional chemokines, prostaglandins, proteases, and growth factors, together with the activation of neutrophils, B cells, and endothelial cells [34]. TNF- $\alpha$  is the main cytokine that initiates inflammation and it is adequate to trigger chronic synovitis, cartilage destruction, and bone erosion [73]. The increased level of TNF- $\alpha$  in the synovium and in synovial fluid of RA patients shows its main action in inflammation and bone degradation [74].

The bioactivities of TNF- $\alpha$  (as in Figure 3) start when it binds to and activates two distinct receptors, TNF-receptor 1 (TNFR1) and TNF-receptor 2 (TNFR2). Both receptors belong to the TNF superfamily receptors and initiate two different intracellular signalling pathways to the transcription of the gene. TNFR1 is exhibited in the body by nearly all cells including the entire lymphoid system, while TNFR2 is mainly expressed in immune cells and mediates the restricted biological reactions but their functional consequences of signalling are not well identified. TNFR1 is universally expressed by all human tissues, has a wide role in the activation of NF- $\kappa$ B, and is the TNF-Alpha major signaling receptor. The adaptor protein TNFR1-associated death domain (TRADD) is recruited by TNFR and causes the accumulation of various complexes. TNFR1-complex II activates the caspase-8 thus inducing the apoptosis. The TRADD protein also activates I $\kappa$ B kinase (IKK) through receptor-interacting protein (RIP) by recruiting the TNF receptor-associated factor (TRAF2). The absence of a death domain in TNFR2 makes it incapable of directly initiating apoptosis. TNFR2 recruits the TNFR-associated factor 2 (TRAF2) to form TNFR2-complex I. This complex activates mitogen-activated protein kinase (MAPKs),

nuclear factor- $\kappa$ B (NF $\kappa$ B), and protein kinase B that initiate inflammation, tissue degeneration, cell proliferation, and cell survival process [72].

TNF regulates downstream cytokines, including IL-6 or IL-1 [72]. Starting in early 1990s, various studies by Feldman and Maini in the 1990s proved that TNF is a main mediator of inflammation which presenting joint damage [34]. The blocking of TNF- $\alpha$  with antibodies in synovial cells from RA patients also significantly decrease the IL-1, IL-6, IL-8, and GM-CSF generation, thus showing higher effect on the inflammation itself compared to the blockade of other high concentration of cytokines present in synovial fluids [75].

#### **f) Interleukin-1-Beta (IL-1 $\beta$ )**

As both TNF- $\alpha$  and IL-1 $\beta$  are one of the main proinflammatory cytokines demonstrated to contribute to RA, both cytokines are pleiotropic which act on different cell types with various biological effects even though the mechanism of it still not yet fully understood [73]. The pleiotropic pro-inflammatory cytokines, IL-1 are cause various diseases, including RA, and are released generally by macrophages, monocytes, and dendritic cells [69]. The three IL-1 family consist of IL-1 $\alpha$ , IL-1 $\beta$ , and IL-1 receptor antagonist (IL-1Ra). IL-1 $\alpha$  is not actively secreted by cells, thereby being released during cell necrosis. IL-1 $\beta$  is an essential part of the host defence mechanism. It is transferred out from the cell where it acts locally or enters the blood circulation. It also secreted upon inflammatory signals during pro-inflammatory activity at the tissue level, leading to vasodilation or activation of innate immune cells such as neutrophils. IL-1 $\beta$  acts on T cells by promoting the differentiation of Th17 and also augments the production of T and B-lymphocytes, prostaglandin E, and the proliferation of fibroblasts [73]. IL-1Ra is articulate in nearly all tissues and the main role is to restrict the uncontrolled activation of IL-1R1 which is involved in IL-1 $\beta$ -mediated inflammation [69]. IL-1 $\alpha$  and IL-1 $\beta$  stimulate the

biological effects while IL-1Ra is an endogenous inhibitor that inhibits the biological actions of the previous two. The binding of IL-1Ra to Interleukin 1 receptor, type 1 (IL-1R1) and Interleukin 1 receptor, type 2 (IL-1R2) does not produce signal transduction because it does not induce the IL-1R accessory protein-interacting domain which is crucial for signal transduction. Plus, it reduces the biological effects of these cytokines by competitively antagonizing the binding of IL-1 $\alpha$  and IL-1 $\beta$ , thereby diminishing cytokines' biological effects. The IL-1R2 acts as a decoy receptor, and binding of IL-1 to this receptor does not induce cell activation due to the very short cytoplasmic domain that is incapable to induce signals [26]. IL-1 $\alpha$  and IL-1 $\beta$  utilized the same receptor and triggered cellular responses by stimulating only a few number of IL-1R1. The ligand binding of IL-1 $\alpha$  and IL-1 $\beta$  to IL-1R1 is enhanced by IL-1R accessory protein (IL-1RAcP). The fully active IL-1R1 binds to the adaptor protein of myeloid differentiation primary response gene 88 (MyD88) that further activates the protein kinase IL-1 receptor-associated kinase (IRAK4). IRAK1 is then activated and phosphorylates by the activated IRAK4. IRAK1 is associated with TRAF6, thus causing the activation of TRAF6 and interacts with a preformed complex of TAK1-TAB1-TAB2. The activated TAK1 will activate the NF $\kappa$ B pathway via IKK activation and MAPK pathways via MAPKK activation, thus resulting in altered gene expression.

Both IL-1 $\alpha$  and IL-1 $\beta$  are engaged in inflammatory reactions but only IL-1 $\beta$  has been found in joint tissues of RA patients [76]. The immune cells shift to the inflammatory site in synovium and communicate with regional synoviocytes, and mesenchymal cells, and this leads to the pro-inflammatory cytokines assembly such as IL-1 $\beta$  and IL-6 [69]. High levels of IL-1 are observed in the synovial fluid and membrane in RA joints [26, 69]. IL-1 also stimulates the production of RANKL and MMP, incorporated in osteoclastogenesis which leads to the destruction

of bone and cartilage degradation [69]. Based on the study by Kay & Calabrese [26] which investigated the efficacy of anakinra, a recombinant human IL-1Ra (r-metHuIL-1ra) to block IL-1 utilization, the blocking of IL-1 has been shown to significantly reduce the symptoms and clinical signs of RA, contrast with placebo. A study by Bergström, et al. [77] also shows the inhibition of cytokine interleukin-1 $\beta$  (IL-1 $\beta$ ) by methotrexate can inhibit the FLS proliferation from the primary FLS samples of RA patients.

#### **g. Interleukin-6 (IL-6)**

Interleukin-6 (IL-6) is released by varieties of cell like T cells, B cells, monocytes, fibroblasts, osteoblasts, endothelial cells, keratinocytes, mesangial cells, and some tumor cells [78]. IL-6 is one of the most expressed cytokines in the rheumatoid synovium [54] and their dysregulation persistent production plays a main part in the characteristic's development of RA [24].

IL-6 promotes B-cell differentiation into plasma cells and produces immunoglobulins. B-cell depletion therapeutic efficacy shows the effects of B-cell activity towards synovial inflammation and joint damage in RA. Neutrophils that express membrane-bound IL-6R are activated by IL-6. The activated neutrophils produce proteolytic enzymes and reactive oxygen intermediates thus destroying tissue and joint damage in RA patients. T lymphocytes are influenced by IL-6 to proliferate and differentiate into TH-17 cells and further produce IL-17 [54, 62].

IL-6 can be activated through both transmembrane IL-6 receptor (tIL-6R) and soluble IL-6 receptor (sIL-6R) (based on Figure 4). The tIL-6R is expressed only on restricted cells such as hepatocytes and some leukocytes, while gp130 is expressed on numerous types of cells. When binding to IL-6R, the complex of IL-6 and tIL-6R associates with signal-transducing molecule gp130 and develops the activation of downstream signaling actions via Janus Kinase (JAK) in the cells of the target and the activation is known as classic signaling pathway. The IL-6

and sIL-6R complex can also bind to gp130 producing the activation of a signalling cascade known as a trans-signalling pathway, which means the sIL-6R has a similar affinity to IL-6 same as tIL-6R. It was suggested that classic signalling is required for regenerative or anti-inflammatory activities, while trans-signalling is required for proinflammatory actions. The inhibition of IL-6 binding leads to disruption of the JAK/STAT system followed by the damping of IL-6 functions in RA including inflammatory and bone-destructive functions. These involve the process of Th17 differentiation, osteoclast differentiation, acute phase response, and matrix metalloproteinases (MMPs) [24, 54].

There is a relationship between the destruction of articular cartilage and MMPs when it is found that IL-6 and CRP correlate with proMMP-3 production in patients with early RA [79], indicating a link between IL-6 and proteinase activity. The blocking of IL-6 trans-signalling by a variant soluble gp130 molecule brings clinical improvement of systemic arthritis, based on the study by Nowell et al. [80] which studied the key role of trans-signalling in RA and used the murine experimental arthritis model. The presence of sIL-6R complex when binding with IL-6 also increased the VEGF levels in cultured synovial fibroblasts of RA patients, proving that high levels of VEGF are correlated with RA disease activity and the appliance of anti-IL-6R antibodies has been significantly lower down the VEGF concentration[81].

#### **Cellular Signalling Pathway in RA**

##### **a) The nuclear factor-KB (NF-KB)**

The existence of activated NF-KB transcription factors has been displayed in human arthritic joints, cultured synovial fibroblasts, and the animal joints by experimentally induced RA. The nuclear factor-KB (NF-KB) pathway activation in the synovial cells of RA patients induces the multitude of responsive genes transactivation that influence the inflammatory phenotype which involves the TNF- $\alpha$  from macrophages, matrix metalloproteinases from synovial fibroblasts and

chemokines that build up the immune cells to inflamed pannus [82].

The events leading to the NF- $\kappa$ B transcription factors activation include the so-called 'classical' or 'canonical' pathway. The result of 'canonical' NF- $\kappa$ B pathway activation which involves heterodimers of p50/p65 suggests that NF- $\kappa$ B may be one of the main regulators of inflammatory cytokine production in RA, based on the existence of both p50 and p65 in the nuclei of cells lining the macrophages and synovial membrane [83]. The action of NF- $\kappa$ B is tightly regulated at multiple levels due to its capability to control the expression of various genes. The three main players in the pathway are the NF- $\kappa$ B transcription factors, inhibitory I $\kappa$ B proteins (I $\kappa$ B, inhibitor of NF- $\kappa$ B), and I $\kappa$ B kinase (IKK) complex, which is a kinase that phosphorylates I $\kappa$ Bs [59].

An important form of the NF- $\kappa$ B response is the formation of dimers that are bound to and inhibited by the I $\kappa$ Bs [82]. The NF- $\kappa$ B transcription factor family in mammals resides of five proteins, p105/p50 (NF- $\kappa$ B1), p100/52 (NF- $\kappa$ B2), p65 (RelA), RelB, and c-Rel that collaborate to create a distinct transcriptionally active homo- and heterodimeric complexes [59]. Three of the family (p65, RelB, and c-Rel) contain carboxy-terminal transactivation domains (TAD). TAD allows the specific dimer to act as an activator or a repressor that cooperates with general transcription factors and co-activators. For example, the presence of a TAD in p65 allows the heterodimer of p50 and p65 to activate the gene transcription. In contrast, the homodimers p50 with no TAD are competing for p50/p65 binding to the NF- $\kappa$ B consensus sequence, which acts as transcriptional repressors [84].

In common resting cells, the NF- $\kappa$ B dimers are associate with one of the prototypical I $\kappa$ B proteins, including I $\kappa$ B $\alpha$ , I $\kappa$ B $\beta$ , and I $\kappa$ B $\gamma$  which regulate their cytosolic localization [59]. Both I $\kappa$ B $\alpha$  and I $\kappa$ B $\beta$  hide the nuclear localization arrangement on the p50/p65 heterodimer by binding to NF- $\kappa$ B, and further inhibit the entry

into the nucleus. IKK complex phosphorylates and degrades I $\kappa$ B $\alpha$  and unmasked the nuclear localization signal thus leads to the active dimer translocation to the nucleus [59]. The IKK complex function in the canonical pathway is to phosphorylate I $\kappa$ B $\alpha$  and I $\kappa$ B $\beta$ , following by marking them for degradation through the ubiquitin/proteasome pathway. The three main subunits covers the canonical IKK complex are IKK1 (known as IKK $\alpha$ ), IKK2 (known as IKK $\beta$ ) and NF- $\kappa$ B essential modulator (NEMO, known as IKK $\gamma$ ) [85]. IKK2 is the most significant to RA, for its catalytic activity is more fundamental for phosphorylation of I $\kappa$ B $\alpha$  by the IKK complex [86].

In the resting state, the dimers are bound with an inhibitory protein (I $\kappa$ B) to prevent the nuclear translocation of NF- $\kappa$ B dimers. The process starts when unknown molecules trigger the reaction together with the surface of T-cell receptors (TCRs) and endogenous or exogenous ligands for the toll-like receptor family (TLR ligands). In the synovium, the resident macrophages are activated, and this leads to I $\kappa$ B phosphorylation by the IKK complex, which is further degraded by the proteasome. This process releases NF- $\kappa$ B dimers and their translocation into the nucleus such as p50/p65 causing the pro-inflammatory cytokines and chemokines expression. Inflammation occurs and large numbers of immune cells are infiltrated into the synovium. FLS (fibroblast-like synoviocytes) synthesize abundant NF- $\kappa$ B-induced genes in response to TNF- $\alpha$  or IL-1, together with chemokines that cause more inflammatory infiltrates and MMPs that develop joint destruction [82].

#### **b) The Mitogen-activated protein kinases (MAPKs)**

Protein kinases are recognized as the enzymes that link covalently to the side chain of specific proteins inside cells; either serine, tyrosine, or threonine [87]. Mitogen-activated protein kinases (MAPKs) belong to the protein kinases family and are regulated by phosphorylation. It catalyzed

the substrate proteins' phosphorylation including other protein kinases, cytoskeletal proteins, transcription factors, and phospholipases [88]. MAPKs are composed of 2 others eventually activated kinases, MAPK kinases (MKKs) and MAPK kinase kinases (MKKKs). In response to different stimuli from the environment, MKKKs are selectively activated. MKKKs phosphorylate and activate specific MKKs, and further selectively phosphorylate specific MAPKs. The phosphates that were transferred to the protein substrate by MAPK during phosphorylation were removed by protein phosphatases [87].

In mammals, MAPKs involved the extracellular signal-regulated kinases (ERKs) and 2 stress-activated protein kinase (SAPKs) families; p38 and c-jun N-terminal kinase (JNK). All of this category of MAPKs has been expressed in the synovial tissue of RA. Much research has focused on MAPK inhibitors due to their implication as the key regulator of pro-inflammatory cytokines production such as IL-1, IL-6, and TNF [89].

The extracellular signal-regulated kinases (ERKs) play their role in the maintenance phase of disease by promoting the formation of pannus. They also play their part in cell division control, in which ERK1 and ERK2 are engaged in the regulation of meiosis, mitosis, and post-mitotic functions in differentiated cells, thus the inhibitors of these enzymes have been studied as anticancer agents [90]. The ERK is found to be activated in synovial fibroblasts and mononuclear cell infiltrates in synovial tissue of RA patients. Therefore, in synovial fibroblasts, ERK is activated during stimulation with IL-1, TNF, and fibroblast growth factor (FGF) [89].

The p38 MAPKs are stimulated by inflammatory cytokines together with other stimuli such as stresses, hormones, and ligands for G protein-coupled receptors in immune cells. They affect human diseases such as autoimmunity and asthma due to their function as key regulators of inflammatory cytokine expression [90].

The JNKs are identified as stress-activated protein kinases based on their activation in response to protein synthesis inhibition [91].

They increased the transcription activity by binding and phosphorylating the DNA-binding protein c-Jun. C-Jun is a main regulator of gene expression and one of the Activator protein-1 (AP-1) transcription complex components [92]. AP-1 regulates various cytokine genes and is activated in return to all stimuli including environmental stress, radiation, and growth factors [93]. JNKs are also important in ruling the apoptosis. Their inhibition elevates the chemotherapy-induced inhibition of tumor cell growth thus indicating they may present as a molecular target for the cancer treatment [90]. IL-1-induced collagenase gene expression involving the JNK-MAPK pathway in synoviocytes and joint arthritis shows that JNK is a relevant therapeutic target for RA [94].

### c) NF-KB and MAPK Relationship

Various studies show the relationship between NF-KB and MAPK to control inflammation-related gene expression in cooperative ways. Studies conducted in human osteoarthritis (OA) chondrocytes and chondrosarcoma cells reveal that TNF- $\alpha$  or IL-1 $\beta$  induce NF-KB and MAP kinases to mediate the RNA/protein expression of MMP1, MMP3, and MMP-13. Both NF-KB and ERK MAPK are activated and mediate MMP-1 gene expression induced by IL-1 in rabbit synovial fibroblasts [89]. These suggest a promising therapeutic approach target to lessen articular cartilage degradation by MMPs in arthritis by the inhibition of TNF- $\alpha$  and IL-1 $\beta$  transduction [57].

### Conclusion

There are various cell types involved in RA pathogenesis have been discussed in this review. Despite the rapid expansion of new members' identification associated with the pathogenesis in recent years, RA remains a complex disease with unknown aetiology beyond a single treatment plan. Recently, it has been discovered that RA is associated with anti-carbamylated protein (anti-CarP) antibodies, also known as anti-

homocitrulline antibodies. Elevations in anti-CarP antibody titres have the potential to worsen existing conditions and accelerate the loss of bone mass. Anti-modified protein antibodies (AMPA), sclerostin, osteoclast and osteoblast co-regulators exert effector functions on immune cells and on bone resorbing osteoclasts, thereby facilitating bone loss. The initiation of innate immune cells develops adaptive immunity, which results in autoantibody production. It is reasonable that fibroblast-like synovial cells (FLS) will remain identified as a significant participator in pathogenesis and might be ultimately expressed as a main therapeutic target. The NF-kB pathway controls the production of pro-inflammatory cytokine and leukocyte recruitment, while MAP kinases play a critical role in key cellular processes that are important for immune system homeostasis. However, further studies on the mediators and sequence of inflammatory cascade should be done to advance of our understanding the damaging part of inflammation which dominates the protective role in RA.

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**Conflict of interest**

None

**Authors contributions**

NSN- draft the preliminary manuscript, literature search, study conception and design. YYK, SFMT, ZNMZ and MNH- supervisory in animal study, in vitro experiments, anti-inflammatory experiments, literature search, review, and edits.

Table 1. The examples of RA-associated genes (*Adapted from Fox<sup>15</sup>*)

<b>Gene</b>	<b>Functions</b>
<b>HLA-DR (shared epitope)</b>	Antigen presentation, lymphocyte activation
<b>PTPN22 (protein-tyrosine phosphatase non-receptor type 22)</b>	Regulation of T cell receptor signalling
<b>PADI4 (peptidyl arginine deiminase 4)</b>	Post-translational change of arginine to citrulline
<b>CCR6 (chemokine receptor 6)</b>	Affinity of Th17 cells to inflammation sites
<b>STAT4 (signal transducer and activator of transcription 4)</b>	Signalling downstream of cytokine receptors



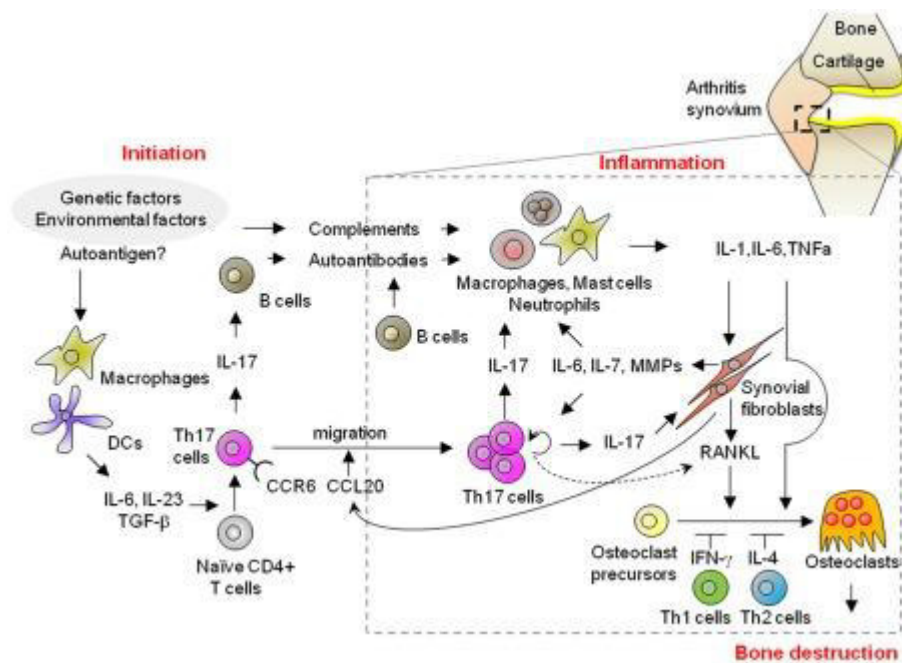


Figure 1. Stages in RA: The mechanism of initiation, inflammatory, and bone destruction.

The RA development is assisted by the role of various types of cell including lymphocytes, innate immune cells, osteoclasts and synovial fibroblasts. In RA synovium, macrophages and synovial fibroblasts stimulate the increased levels of the proinflammatory cytokines IL-1, IL-6, and TNF- $\alpha$ . These proinflammatory cytokines directly and indirectly generate the other proinflammatory cytokines and chemokines along with matrix-degrading enzymes, thus developing cytokine “storm” in the inflamed synovium. The interplay between CD4+ T-cells and mesenchymal cells in joints also play important role in the inflammation and bone destruction phases (*Adapted from Komatsu & Takayanagi<sup>20</sup> with permission*).

Table 2. Three mechanisms in synovial hyperplasia (*Adapted from Adam Mor<sup>28</sup>*)

Mechanism	Process
<b>1. Hyperproliferation of FLS</b>	Several growth factors are overexpressed in the rheumatoid joint including; a) Platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) b) MLS secrete transforming growth factor-h (TGF-h) which is overexpressed in RA synovium.
<b>2. Decreased apoptosis</b>	RA-SF express high levels of Bcl-2 which is a proto-oncogene on chromosome 18q that inhibit apoptosis via inhibition of caspase activation.
<b>3. Decreased senescence</b>	Senescence occurs when the ability of aging cells to continue to divide is limited but it is still alive and enter a permanently non-proliferative state.

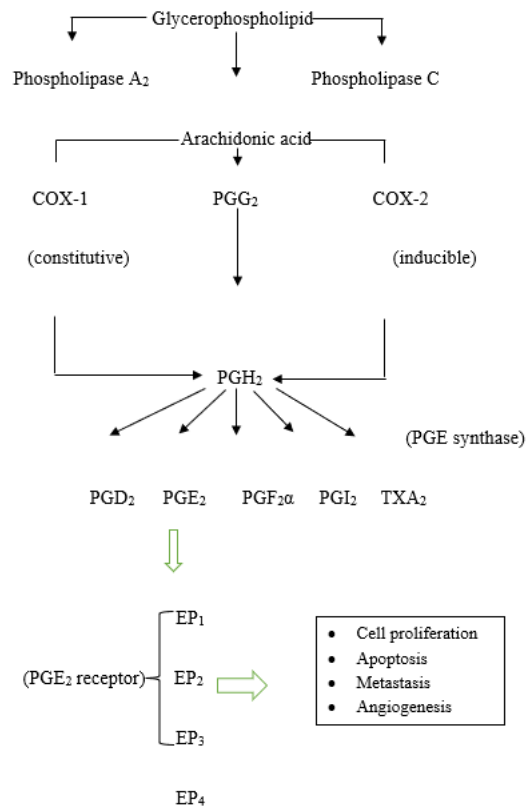


Figure 2. The conversion of arachidonic acid to PGE<sub>2</sub> in the biosynthesis of PGE<sub>2</sub> and the involvement of COX-2 in the disease development.

Arachidonic acid is released by phospholipase A2 from the membrane phospholipid. Enzymatic reactions by COX-1 and COX-2 converts the Arachidonic acid to prostaglandin (PG)-G2 and further the conversion into PGH2. The PGE2 is converted from PGH2 which catalysed by PGE2 synthase (PGES) with other PG isoforms. PGE2 is the major prostanoid among them and display various biologic activities via its EP receptors. (*Adapted from Urade<sup>63</sup> with permission*).

Table 3. The main roles of cytokines in (*Adapted from Nalbant & Birlik<sup>69</sup>*)

<b>Cytokines</b>	<b>Functions</b>
<b>1. Pro-inflammatory cytokines</b>	
<b>TNF-alpha</b>	Autocrine stimulator and paracrine inducer of other inflammatory cytokines. Stimulate fibroblasts to express adhesion molecules such as intracellular adhesion molecule 1 (ICAM-1). Induces IL-1 production. (Vasanthi et al., 2007)
<b>IL-1</b>	Up-regulate the expression of CAMs. Stimulate production of chemokines. Stimulate synoviocytes and chondrocytes to release MMPs and other proteinases. Trigger the differentiation of osteoclasts. Activate mature osteoclasts to erosion. Stimulates TNF production.
<b>2. Inflammatory cytokines</b>	
<b>TNF-alpha &amp; IL-1</b>	(as the above)
<b>IL-6</b>	Stimulate neutrophil migration. Inducing neovascularization, infiltration of inflammatory cells, and synovial hyperplasia Induce cartilage degeneration by producing matrix metalloproteinases (MMPs) (Hashizume & Mihara, 2011)
<b>IL-15</b>	Recruit and activate T cells. Activate neutrophils. Promotes synovial cytokine (including TNF) and chemokine. (Baslund et al., <sup>70</sup> )
<b>IL-16</b>	Anti-inflammatory effect by regulation of Tregs. Activate expression of adhesion molecules and recruit leukocytes to involved joints (Yoshida & Tanaka, <sup>24</sup> ).
<b>IL-17</b>	Increase local chemokine production.

	Increase IL-6 production. Promotes the effect of IL-1b, TNF-a and IFN-g (Nalbant & Birlik, <sup>69</sup> ).
<b>IL-18</b>	Increase the production of fibroblast-like synoviocytes and chondrocytes (Dai et al. <sup>71</sup> ).
<b>IL-21</b>	Activate TH17 cells.
<b>IFN-gamma</b>	Immune modulation (both protection and activation).
<b>Granulocyte – macrophage – colony stimulating factor (GM-SF)</b>	Promotes existing RA.
<b>3. Anti-inflammatory cytokines</b>	
<b>IL-10</b>	Inhibit Th1 cell activity by suppressing IFN- $\gamma$ expression. Protection against cartilage destruction combination with IL-4.
<b>IL-4</b>	Increased level in synovial fluid during only synovial inflammation.
<b>IL-13</b>	Synergistic or inhibitory roles during the arthritis with IL-10, IL-21R, galectin-3 and TGF $\beta$ .
<b>IL-20</b>	Regulates osteoclast differentiation.
<b>4. Natural cytokines antagonist</b>	
<b>IL-1 receptor antagonist (IL-1ra)</b>	Low levels of IL-1 receptor antagonist (IL-1ra) causes erosive disease in patients.
<b>Soluble type 2 IL-1 receptor</b>	Cause competitive inhibition by binding interleukin-1 $\alpha$ (IL1A), interleukin-1 $\beta$ (IL1B) and interleukin 1 receptor antagonist (IL1Ra).
<b>Soluble TNF receptor (sTNF-RI)</b>	It is not well-known; possible effect is to cause cleavage of TNF alpha.
<b>IL-18 binding protein</b>	Protect against the joint destructive effect by binding IL-18 in RA.

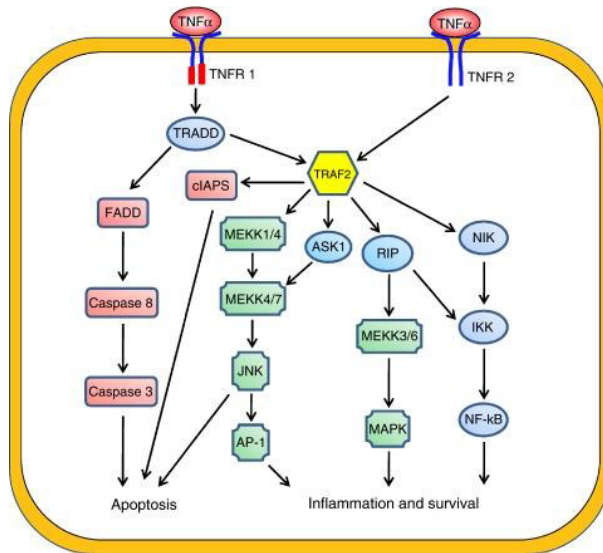


Figure 3. The activities of TNF initiated by the binding and the activation of two different receptors, TNF- receptor 1 (TNFR1) and TNF-receptor 2 (TNFR2) (*Adapted from Noack, & Miossec<sup>72</sup> with permission*).

Table 4. TNF- $\alpha$  action on different cells in rheumatoid arthritis (*Adapted from Vasanthi et al.,<sup>75</sup>*)

<b>Cell type</b>	<b>TNF-<math>\alpha</math> action</b>
<b>Macrophages</b>	Increases proliferation and production of cytokine.
<b>Activated T-cell</b>	Enhances proliferation and increases interleukin (IL)-2 receptor.
<b>B-cell</b>	Increases proliferation and differentiation.
<b>Synovial lining cell</b>	Induces proliferation, induces synthesis of IL-1, granulocyte monocyte-colony stimulating factor (GM-CSF), stromelysin, collagenase and prostaglandins.
<b>Endothelial cells</b>	Induces expression of intracellular adhesion molecule 1, vascular cell adhesion molecule 1 (VCAM-1), endothelial leucocyte adhesion molecule-1 (ELAM-1) and IL-8.

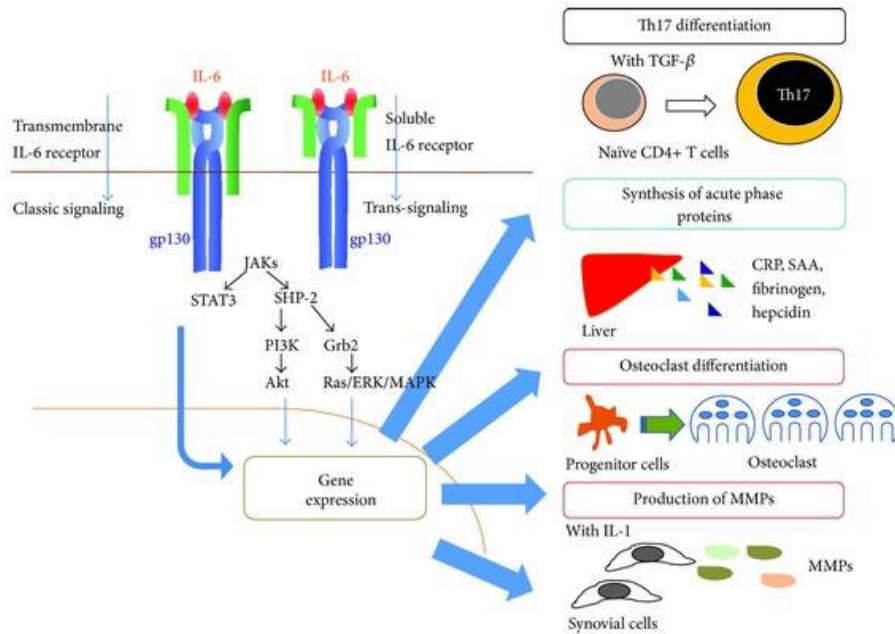


Figure 4. IL-6 signalling pathway. The pleiotropic activity of IL-6 begins with gp130 by binding to transmembrane or soluble IL-6 receptor. This activates the IL-6 signalling pathway and the STAT3 transcriptional factors initiate numerous gene expressions, producing cell differentiation or proliferation (JAKs, Janus kinases; STAT3, signal transducer and activator of transcription 3; SHP-2, SH2 domain-containing tyrosine phosphatase 2; PI3K, phosphoinositol-3 kinase; Grb2, growth factor receptor-bound protein 2; ERK, extracellular signal-regulated kinase; MAPK, mitogen activated protein kinase; Akt, protein kinase B; TGF- $\beta$ , transforming growth factor beta; CRP, C-reactive protein; SAA, serum amyloid A; MMPs, matrix metalloproteinases) (*Adapted from Yoshida & Tanaka,<sup>24</sup> with permission*)



Table 5. The NF- $\kappa$ B transcription factors (RA, rheumatoid arthritis; TAD, transactivation domain)

*(Adapted from Simmonds & Foxwell,<sup>82</sup>)*

<b>Protein</b>	<b>Gene</b>	<b>Function</b>
<b>p105/p50</b>	NFKB1	Canonical pathway p105 constitutively processed to p50 p50/p65 form activating heterodimers p50/p50 form inhibitory homodimers Found in RA synovium
<b>p100/p52</b>	NFKB2	Non-canonical pathway p100 processing to p52 tightly regulated
<b>p65</b>	RfgGHELA	Canonical pathway p50/p65 activating heterodimers Contains TAD Found in RA synovium
<b>RelB</b>	RELB	Contains TAD
<b>c-Rel</b>	REL	Contains TAD

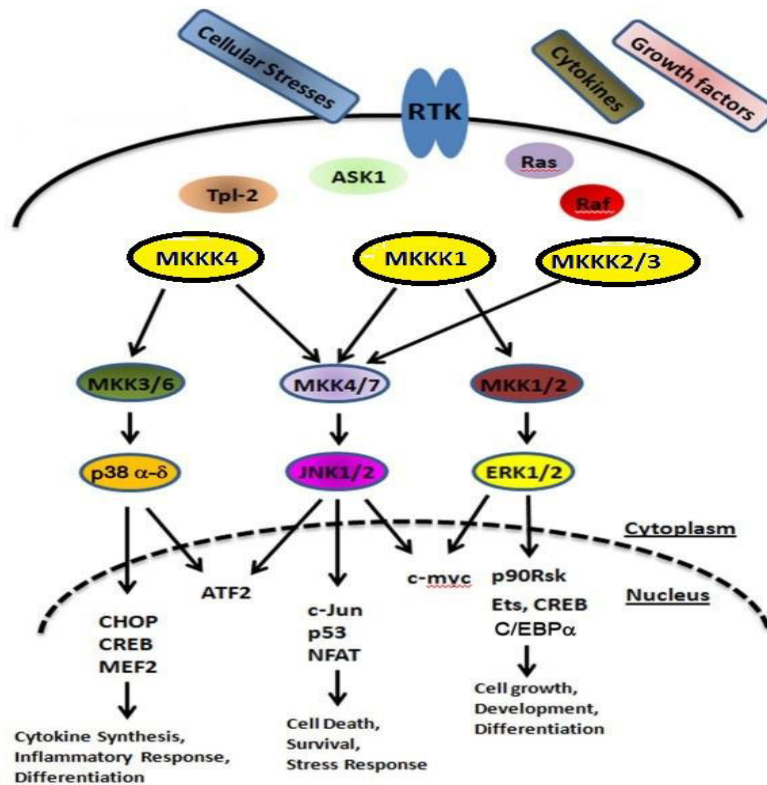


Figure 5. Mammalian MAPK signalling cascades: simplified diagram depicting the MAPK signaling network. MAP kinase pathways involved 3 basic parts that initiated by a signal from an external stimulus. MKKKs activates MKKs by dual phosphorylation which then activates a MAPK by the same mechanism. MKKKs, RAFs, and Tpl2 initiate the basic ERK pathway which activates MKK1 and MKK2, which in turn activates the multiple substrate by ERK1 and ERK2 phosphorylation. MKKKs activate MKK3 and MKK6 and activate 4 isoforms of p38. In the JNK pathway, these MKKKs activate MKK4 and MKK7, followed by JNK1 activation. All the pathways of p38, JNK and ERK further activate multiple transcription and translation factors (Adapted from Munshi & Ramesh,<sup>95</sup> with permission).

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## REVIEW ARTICLE

# Exercise and Hypertension: A Review on Exercise Recommendation, Mechanism of Action, Exercise-Related Risk and Innovative Exercise Approach.

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### Abstract

Hypertension, commonly known as high blood pressure, is a condition in which the force of blood pushing against the walls of the arteries is consistently higher than normal. This condition can lead to serious health problems such as heart disease, stroke, and kidney disease. There are several strategies that individuals with hypertension can adopt to manage their condition and engaging in exercise is among the most effective non-pharmacological approaches. This study examines the critical role that exercise plays in controlling hypertension, a condition that is heavily impacted by stress and modern sedentary lifestyles, underscoring the need for efficient intervention measures. The paper explains biological mechanisms supporting blood pressure regulation while highlighting the advantages of consistent physical activity, including reduced blood pressure and improved cardiovascular health, through a variety of exercise modalities, including aerobic activities, resistance training, high-intensity interval training (HIIT), and circuit training (CT). These include a decrease in inflammatory markers, an improvement in autonomic balance, and vasodilation. The review also covers the significance of tailoring exercise regimens to each patient's unique health profile to maximise benefits and minimise dangers. Exercise planning is important for people with hypertension since it affects their blood pressure. Factors to consider include the intensity, duration, and any interactions with medications. Innovative exercise approaches, particularly HIIT and CT, are examined for their effectiveness in cardiovascular improvement and hypertension management. Conclusively, the document advocates for integrating exercise into hypertension management and prevention, underscoring its pivotal role in enhancing patient health outcomes and quality of life in a comprehensive care strategy.

**Keywords:** *Hypertension, exercise, exercise-related risk, innovative exercise approach physical activity.*

## Introduction

In today's fast-paced world, where stress and sedentary lifestyles prevail, the prevalence of hypertension, commonly known as high blood pressure, has reached alarming levels [1]. Hypertension poses a significant threat to global health, increasing the risk of heart disease, stroke, and other cardiovascular complications [2]. Fortunately, amidst this health crisis, exercise emerges as a potent tool for combating hypertension and promoting overall well-being. Hypertension is classified when Systolic Blood Pressure (SBP) is more than 140 mmHg and/ or Diastolic Blood Pressure (DBP) is more than 90 mmHg. Blood Pressure (BP) should be taken more than two times and space of 1 to 2 minutes apart in more than two occasions. The average reading will determine the level of BP [3]. Prehypertension is diagnosed when SBP is between 120 mmHg to 139 mmHg and DBP is between 80 mmHg to 89 mmHg [4]. Hypertension, characterized by elevated blood pressure levels, arises from a variety of factors, including genetics, diet, obesity, and lack of physical activity [5]. It silently creeps into our lives, often unnoticed, gradually damaging our arteries and putting undue strain on the heart [6]. However, research over the years has unequivocally demonstrated the positive impact of exercise in managing and preventing hypertension [7].

Engaging in regular physical activity not only reduces blood pressure but also improves cardiovascular health in numerous ways [8]. Exercise promotes vasodilation, which widens blood vessels, enhancing blood flow and reducing resistance [7]. Additionally, it helps strengthen the heart muscle, making it more efficient in pumping blood, and improves overall blood lipid profiles, reducing the risk of atherosclerosis and plaque build-up [8].

The type, intensity, and duration of exercise play crucial roles in effectively managing hypertension [8]. Aerobic exercises, such as brisk walking, jogging, swimming, and cycling, have been widely recommended by healthcare professionals [7]. These activities stimulate the

heart and lungs, increasing oxygen supply to the body and promoting cardiovascular fitness. Resistance training, involving weights or resistance bands, can also contribute to blood pressure management by improving muscle strength and enhancing metabolic rate [8].

Furthermore, exercise not only benefits physical health but also has a profound impact on mental well-being. Regular physical activity releases endorphins, the "feel-good" hormones, reducing stress, anxiety, and depression [9]. By promoting better sleep patterns and boosting self-esteem, exercise creates a positive feedback loop that supports overall health and reduces the risk of hypertension. It is important to note that individuals with hypertension should consult their healthcare providers before embarking on an exercise regimen. Healthcare professionals can provide personalized recommendations, considering the individual's health status, age, and any existing medical conditions [7].

In short, hypertension poses a significant global health challenge, but exercise offers a powerful countermeasure. Engaging in regular physical activity can help to manage blood pressure levels, improve cardiovascular health, and enhance overall well-being [8]. By incorporating exercise into our daily routines, we can take proactive steps towards a healthier heart and a better quality of life.

Given the well-established benefits of exercise in managing hypertension, there is a crucial need to develop specific exercise prescriptions tailored to hypertensive patients based on the FITT (Frequency, Intensity, Time, and Type) principle. This review aims to fill that gap by providing detailed guidelines on the most effective exercise regimens for this population. By focusing on personalized exercise plans that consider the unique needs and limitations of individuals with hypertension, the study seeks to optimize cardiovascular outcomes and overall health. Understanding the ideal combinations of exercise frequency, intensity, duration, and type will empower healthcare professionals to offer more targeted and effective interventions, thereby

improving patient adherence and long-term management of hypertension.

## Discussion

### Hypertension in Malaysia

Hypertension has emerged as a significant global health challenge, contributing to a rise in chronic diseases such as stroke, myocardial infarction (MI), other heart diseases, and renal problems. It is estimated that approximately 6% of global deaths are attributed to hypertension [10]. Research reveals that among cardiovascular patients in 21 regions globally, complications of hypertension have led to the death of 9 million individuals. Furthermore, it has contributed to 6.7 million deaths from stroke and 7.4 million deaths from coronary heart disease [11]. The World Health Organization (WHO) predicts that by the year 2025, nearly 29.2% of the world's population will be affected by hypertension [12]. WHO also emphasizes the disparities in the prevalence of hypertension between low- and middle-income countries, which can be attributed to weak healthcare systems. Additionally, variations in the number of hypertension cases exist within regions and subgroups of populations within the same country [13].

In Malaysia, the prevalence of hypertension remains high with limited improvement among the population, despite the implementation of several government policies related to hypertension [14]. The incidence of hypertension increased by 0.7% from 34.6% in 2006 to 35.3% in 2015 [10]. National Health and Morbidity Survey (NHMS) [15] conducted across different states in Malaysia has revealed that Perak has the highest prevalence of hypertension, which is 22.7%, followed by Negeri Sembilan (19.8%) and Sarawak (19.6%). On the other hand, the lowest prevalence of hypertension in 2019 was observed in Selangor with the prevalence of 12.7% [15].

Analysis of surveys conducted by the National Health and Morbidity Survey in 2006, 2011, and 2015 indicates that the number of hypertension cases was higher in rural areas compared to urban

areas [13]. Over the past decade, there has been no significant change in the distribution of cases between genders. Moreover, the surveys have shown that the prevalence of hypertension is higher among the population aged above 60 years compared to the younger population. Additionally, there is a higher incidence of hypertension among individuals with a Body Mass Index (BMI) higher than 30.0 kg/m<sup>2</sup>, which falls under the obese category [36].

Examining the ethnic distribution of hypertension cases in Malaysia, Chinese individuals had the highest number of cases in 2006; however, the number of cases reduced in 2015 [10]. In 2019, Bumiputra Sarawak ethnicities had the highest prevalence of hypertension, followed by Indian, Chinese, and Bumiputera Sabah populations [15]. Another study conducted by Naidu et al. [12] reported that the prevalence of hypertension is slightly higher in men compared to women, with an increase in the older population and a higher incidence among households with lower incomes [12].

The prevalence and severity of hypertension in Malaysia vary across different groups. Prehypertension, a precursor to hypertension, is more prevalent in men compared to women. Additionally, certain ethnicities such as Baba, Sikh, Eurasian, Chitty, and foreigners, as well as individuals with no formal education, smokers, married adults, and those with a BMI over 25 kg/m<sup>2</sup>, show a higher prevalence of prehypertension [12].

Regarding stage 1 hypertension (SBP: 130-139 mmHg; DBP: 80-89 mmHg), is more prevalent among men, individuals residing in rural areas, those of Malay ethnicity, those with lower incomes, less education, widowed individuals, and those with underlying chronic diseases such as diabetes Mellitus (DM), high cholesterol levels, and abdominal obesity. In contrast, stage 2 hypertension (SBP: >140 mmHg; DBP: >90 mmHg) is more prevalent in women compared to men. The study also reveals a higher number of cases among individuals living in rural areas, those of Malay ethnicity, individuals with lower

education levels, widowed individuals, those with lower incomes, non-smokers, and individuals with underlying DM, abnormal cholesterol levels, and obesity [12].

### **Exercise recommendation for hypertension**

Practicing a healthy lifestyle is an effective method for controlling BP. Incorporating healthy habits such as engaging in a daily exercise program, preventing smoking, maintaining an ideal body weight, following a balanced diet, and reducing sodium intake can contribute to BP control [16]. Exercise is well-known for its positive effects on BP regulation. However, it is crucial to select the appropriate exercise modalities and prescriptions, as they can yield different effects on individuals [17].

Aerobic exercise (AE) such as running, swimming and cycling is a recommended modality for reducing BP and has demonstrated effectiveness in treating chronic conditions like diabetes mellitus (DM), hypertension, cardiovascular disease (CVD), and coronary artery disease [3]. Chulvi-Medrano, Sanchis-Cervera, Tortosa-Martínez, and Cortell-Tormo [18] also support the use of AE as a primary modality for the prevention, treatment, and control of BP. According to the American College of Sports Medicine (ACSM) [19] guidelines, AE should be performed for at least 5 days a week, with each session consisting of at least 30 minutes of moderate-intensity exercise, or alternatively, at least 3 days a week with at least 20 minutes of high-intensity exercise. However, it is essential to tailor the exercise prescription to the individual needs of hypertension patients to achieve optimal effects [20].

Additionally, dynamic resistance exercise is another modality that can be utilized for the treatment of hypertension. Individuals who engage in this type of exercise can experience a reduction in their SBP by up to 1.8 mmHg and their DBP by up to 3.3 mmHg [37]. The recommended protocol for dynamic resistance exercise includes 2 to 3 sessions per week at a

moderate intensity of 60% to 80% of 1 Repetition Maximum (1RM), involving 8 to 10 exercises targeting major muscle groups, with 10 to 12 repetitions and 2 to 3 sets for each muscle group [18]. Other studies suggest that exercising 3 days a week with low to moderate intensity at 60% to 65% of 1 RM, performing 10 to 12 repetitions and 3 sets of exercises, can lead to a reduction of resting blood pressure by as much as 6 mmHg [21]. The most significant benefits of dynamic resistance exercise are typically observed within 6 to 48 weeks of regular exercise sessions.

Furthermore, recent studies have proposed isometric resistance exercise as a potential treatment for hypertension patients. Unlike dynamic resistance exercise, isometric exercise involves muscle contraction without affecting the range of joint motion [3]. Some studies have demonstrated that isometric exercise provides greater benefits in lowering blood pressure compared to other forms of training. The recommended protocol for isometric exercise included 3 to 4 sessions per week, with 4 repetitions of 20% to 50% of maximal voluntary contraction, holding for 2 minutes and resting for 1 to 4 minutes between repetitions, for 4 to 10 weeks of exercise sessions [22]. Another study recommended performing 4 sets of 2 minutes of isometric exercise with 2 minutes of rest between trials, 3 times a week for 4 weeks, which effectively lowered blood pressure levels [23]. Reductions in blood pressure have also been observed in male patients who performed unilateral arm exercises during 8 weeks of isometric exercise sessions [24]. Unilateral exercise has shown a greater reduction in blood pressure compared to bilateral exercise, and the effect is more pronounced in individuals aged 45 years and above. Studies have also indicated that upper limb isometric exercise provides more benefits compared to lower limb exercise [3].

Combining aerobic exercise (AE) and dynamic resistance training on the same day or alternate days can also be beneficial for individuals with hypertension. This combination has been shown to lower blood pressure by approximately 3



mmHg [25]. Such exercises are also recommended by the ACSM where the exercise prescription involves performing AE on all days of the week at a moderate intensity of 40% to 60% of oxygen uptake reserve (VO<sub>2</sub>R), along with 2 to 3 days of moderate-intensity resistance training at 60% to 80% of 1RM, with 2 to 3 sets of 8 to 12 repetitions [3]. In older adults, a study has shown that a 6-month combined exercise protocol can lead to reductions in SBP and DBP. The exercise frequency is 3 times a week, with a moderate intensity ranging from 50% to 80% of 1RM for resistance training, 60% to 90% of maximum heart rate for aerobic training, and exercise durations ranging from 40 to 90 minutes per session [26].

The summary of various exercise modality recommendations for individuals with hypertension is presented in Table 1. These recommendations encompass aerobic exercise (AE), dynamic resistance exercise, isometric resistance exercise, and a combination of AE and dynamic resistance training. Each exercise modality specifies the description or types, frequency, intensity and duration.

### **Mechanism of exercise in improving hypertension**

There are various mechanisms involved in controlling and treating hypertension. Aerobic exercise (AE) has been shown to lower blood pressure by reducing signals to the sympathetic nervous system, leading to vasodilation of blood vessels [27]. Prolonged AE among hypertension patients can cause remodelling of the left ventricle of the heart without chamber enlargement, resulting in reduced oxidative stress, inflammation, and improved autonomic system function [28].

Additionally, AE has a significant impact on body weight reduction, particularly in fat quantity [25]. A 5% reduction in body weight can help to regulate the renin-angiotensin-aldosterone system, which plays a crucial role in blood pressure regulation. Weight loss also contributes

to the repair of kidney injuries, thereby assisting in blood pressure control [28].

During dynamic resistance exercise, there is an initial increase in blood pressure due to the response of the cardiovascular center in the brain (medulla oblongata) when muscles contract. The contraction of muscles raises intramuscular pressure, affecting blood flow in the arteries. To counteract this, the brain signals an increase in blood pressure to ensure adequate muscle perfusion [18]. The effects of resistance exercise become evident a few hours later, characterized by a decrease in norepinephrine, angiotensin II, endothelin, and adenosine. Reduced receptor sensitivity to vasoconstriction mediators in the central nervous system (CNS) contributes to decreased pulmonary vascular resistance (PVR) and improved baroreflex sensitivity. Nitric oxide and prostaglandins released after exercise also promote vasodilation of blood vessels [18].

Isometric resistance exercise affects blood pressure through various mechanisms. Exercise substantially enhances sympathetic vasoconstrictor outflow, aiding in the redistribution of cardiac output to the working muscles. This heightened sympathetic vasoconstrictor activity also limits the rise in blood flow to the contracting muscles during exercise, helping to maintain blood pressure [38]. Isometric handgrip exercise also increases blood flow to other tissues in the body. These effects are related to the alteration of blood vessel resistance, an essential factor in blood pressure regulation. Furthermore, isometric exercise may reduce oxidative stress and the amount of oxygen radicals that contribute to increased blood pressure [29].

Combination exercises, which involve both AE and dynamic resistance training, have been shown to lower blood pressure by reducing baroreflex response and modulating the autonomic nervous system. In hypertension patients, this combination exercise reduces inflammation in the cardiac and renal systems [25]. Moreover, combination exercise reduces oxidative stress-related damage to the heart and kidneys by

decreasing protein oxidation and lipoperoxidation [30]. A four-week program of combination exercise has been shown to improve blood vessel health, promoting post-exercise vasodilation and reducing arterial stiffness or atherosclerosis, which can contribute to elevated blood pressure [31].

### **Limitations and exercise-related risk for hypertensive patients**

Exercise offers numerous benefits for individuals with hypertension, but it is crucial to ensure proper execution to avoid worsening the condition. AE yields maximum benefits when performed at moderate to high intensity, necessitating supervision of therapist to ensure correct intensity. Without therapist supervision and adherence to the prescribed exercise plan, the reduction in blood pressure may be minimal, leading to poor long-term outcomes and exercise discontinuation [32]. The duration of exercise also plays a significant role, with ACSM recommending at least 30 minutes a day, which may be challenging for individuals with busy schedules, as shorter durations yield minimal benefits for hypertension patients [18].

Similarly, dynamic resistance exercise requires adequate preparation before, during, and after the workout. Different equipment can be used, including weight machines, free weights, body weight, or resistance bands. It is important to be cautious when using free weights due to the involvement of coordinated movements, as incorrect execution can lead to injuries. Holding one's breath while lifting weights can also increase blood pressure, emphasizing the importance of proper breathing techniques [4]. Additionally, selecting the appropriate weight is essential, typically ranging from 60% to 80% of the individual's one-repetition maximum (1RM) or based on the Rating Perceived Exertion (RPE) using the Borg Scale. Inadequate weight may not elicit the desired response, while excessive weight can dramatically increase blood pressure during exercise [3].

Isometric strengthening exercise carries a high risk of elevating blood pressure. The protocol for this type of exercise involves constant muscle contraction and rest intervals. However, the recommended hold and rest durations may not be suitable for all hypertension patients, as some individuals may require longer recovery periods. Insufficient recovery time can contribute to increased blood pressure levels [23]. Additionally, patients tend to involuntarily hold their breath during exercise, triggering the Valsalva manoeuvre and further elevating blood pressure [4]. Proper monitoring of isometric strengthening exercise in high-risk hypertension patients is crucial due to limited research on its safety and effectiveness for this population [23].

Combining aerobic and resistance training may lead to a slight increase in weight due to changes in body composition, such as a reduction in total body fat percentage but it elicits beneficial changes in body composition in terms of increased lean body mass [25]. Hypertension patients participating in sports events with weight classifications should be aware of this potential weight increase. It is worth noting that regular exercise can result in lowering blood pressure levels, but in some cases, individuals taking medications like alpha blockers or vasodilators may experience abrupt reductions in blood pressure after exercise, increasing the risk of fainting. Diuretic medications can affect the thermoregulatory system, causing heat intolerance and reducing exercise duration [33]. Overall, exercising with hypertension requires careful attention to various factors, including intensity, duration, technique, and medication management, to ensure safety and maximize the benefits of exercise for blood pressure control.

### **Innovative exercise approaches for hypertensive populations**

First and foremost, high-intensity interval training (HIIT) is well-known for its benefits in improving cardiorespiratory fitness and muscle strength in the general population. HIIT involves alternating between high-intensity aerobic

exercise at 85% to 90% of maximum oxygen consumption ( $VO_{2max}$ ) and periods of low-intensity exercise or rest [32]. Recently, HIIT has been utilized in the treatment of hypertension patients. High-intensity exercise stimulates the sympathetic system and elicits greater adaptations in the body. Additionally, it improves endothelial function, which is crucial for regulating atherosclerosis. Research indicates that HIIT produces similar results of continuous aerobic exercise performed at 60% to 65% of  $VO_{2max}$  in terms of Post Exercise Hypotension (PEH). A 12-week HIIT protocol at 85% to 90% of  $VO_{2max}$  has been shown to reduce blood pressure, enhance cardiac function, and lower mean heart rate more effectively than continuous aerobic exercise performed at 60% to 65% of  $VO_{2max}$  [18]. Another study demonstrated that HIIT, consisting of four intervals of high-intensity exercise (85% to 90% of Peak Heart Rate) for four minutes each, with three minutes of recovery phase at 50% of Peak Heart Rate, performed three times a week for one month, led to improvements in diastolic function and peak oxygen consumption [34].

Next, Circuit Training (CT) is another innovative modality for treating hypertension patients. CT involves strength exercises targeting major muscle groups using light loads and higher repetitions. The CT program typically includes several stations, with patients moving from one station to another after completing 8 to 20 repetitions with different major muscle involvement. The rest period between stations ranges from 15 to 30 seconds, depending on the patient's fitness level [35]. A CT program consisting of six stations of strength exercises performed three times a week for one month has shown improvements in vascular function and a reduction in blood pressure levels [31].

In clinical settings, exercise progression should be adjusted based on the patient's fitness level. Progression should be gradual and not abruptly increase exercise intensity, as this can lead to an increase in blood pressure and a higher risk of injury [18]. Patients who are not accustomed to

the new exercise intensity may experience fatigue and slower recovery, which can disrupt their exercise program [22]. Therapists should also consider the medications patients are taking, as changes in antihypertensive drugs during an exercise program can affect their ability to exercise [32]. Finally, warm-up before exercise and cool-down after exercise sessions are crucial for hypertension patients. Warm-up prepares the body for exercise and helps to prevent a drastic increase in blood pressure during exercise. Cooling down is essential to prevent a rapid decrease in blood pressure, especially in patients taking medications such as alpha-blockers [33].

## Conclusion

In conclusion, exercise plays a significant role in the management and treatment of hypertension. Various forms of exercise, such as aerobic exercise, dynamic resistance training, HIIT and CT, have been shown to effectively lower blood pressure, improve cardiovascular fitness, and enhance overall health in hypertension patients. Aerobic exercise helps lower blood pressure by reducing sympathetic nervous system activity and promoting vasodilation of blood vessels. It also aids in weight loss, which can further contribute to blood pressure control and improve kidney function. Dynamic resistance exercise, although initially causing a temporary increase in blood pressure, leads to long-term benefits by improving autonomic system function, and by reducing inflammation and oxidative stress.

HIIT with its alternating high-intensity and low-intensity intervals, offers a time-efficient approach to improving cardiorespiratory fitness and reducing blood pressure levels. CT, on the other hand, focuses on strengthening major muscle groups and has shown positive effects on vascular function and blood pressure reduction. For the future studies, researcher might also examine the role of individualized exercise prescriptions based on genetic factors, age, and comorbidities, to better tailor exercise interventions for hypertension patients. Not to

forget, understanding the barriers to exercise adherence and developing strategies to overcome them could enhance the effectiveness of exercise as a therapeutic tool in hypertension management. By incorporating regular exercise into their lifestyle, hypertension patients can significantly improve their blood pressure control, reduce the risk of cardiovascular complications, and enhance their overall well-being. It is important for healthcare professionals to emphasize the importance of exercise in hypertension management and provide appropriate guidance to ensure safe and effective implementation.

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**Authors contribution**

Wan Muhammad Ihsan Wan Nawi and Mohamad Hafiz Abu Seman: conceptualization of research, drafting and writing the manuscript; Nurshazana Akmal Jamaludin and Rabiatal Adawiah Abdul Rahman: method analysis and editing manuscript; Muhammad Iqbal Shahrudin and Nur Ainah Mohd Shipah: final checking and editing manuscript.

Table 1. Summary of exercise modality recommendation for hypertensive population.

Exercise Modality	Frequency	Intensity	Duration	Type or Description
Aerobic Exercise (AE)	At least 5 days a week, or alternatively, at least 3 days a week	Moderate to high intensity	At least 30 minutes for moderate intensity, or at least 20 minutes for high intensity	Running, swimming, cycling
Dynamic Resistance Exercise	2 to 3 sessions per week	60% to 80% of 1 Repetition Maximum (RM)	8 to 10 exercises targeting major muscle groups, with 10 to 12 repetitions and 2 to 3 sets for each muscle group	Free weights or machine weights
Isometric Resistance Exercise	3 to 4 sessions per week	20% to 50% of maximal voluntary contraction	4 repetitions of 20% to 50% of maximal voluntary contraction, holding for 2 minutes and resting for 1 to 4 minutes between repetitions	Muscle contraction without joint motion (isometric handgrip exercise)
Combination of AE and Dynamic Resistance Training	5 to 7 days of AE; 2 to 3 days of resistance training	Moderate intensity of AE; 60% to 80% of 1RM for resistance training	At least 30 minutes for AE; 2 to 3 sets for each muscle group for resistance training	Running, swimming or cycling for AE; machine or free weight for resistance exercise

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## REVIEW ARTICLE

# Effectiveness of Face Masks in Preventing the Transmission of Respiratory Tract Infections: A Rapid Review.

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### Abstract

**Introduction:** People wear various types of face masks to protect themselves from breathing in dust, pollutants, allergies, and harmful organisms. In light of the recent Covid-19 pandemic, mask-wearing has been made mandatory globally. As part of personal protective equipment and a public health strategy to stop the spread of illnesses, wearing face masks has been widely recommended. This rapid review was conducted to explore and analyze the effectiveness of face mask usage in preventing the transmission of respiratory tract infection. **Method:** The review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Twenty three out of 134 articles met the inclusion criteria. The types of masks studied were N95 masks, surgical masks, cloth masks, non-medical masks, and unspecified face masks.

**Result and conclusion:** The analysis of the review indicates that the usage of masks does indeed offer a certain degree of protection to the individual and to those around them. Therefore, policy makers should encourage the general population to use face masks for health protection. However, more evidence is required in terms of clarifying the usage of the mask in various settings and against diverse types of infection. It is emphasised that the use of face masks, in combination with other preventive measures such as handwashing and social distancing, should be implemented concurrently to prevent any outbreak of respiratory tract infections.

**Keywords:** *Effectiveness, face masks, N95 mask, surgical mask, transmission, respiratory tract infection.*

## Introduction

In recent years, many infections have endangered people, causing acute respiratory illnesses that have a negative impact on human lives and civilizations. Respiratory tract infections have always resulted in significant morbidity and mortality globally. It is one of the most common problems faced by individuals regardless of age and gender. Respiratory tract infections can be caused by a wide variety of pathogens such as viruses or bacteria. Although most of the infections are usually mild and not incapacitating, some respiratory tract infections have the capability to pose a serious problem in cases of pandemics or even epidemics. This is mainly due to the high transmissibility rate among humans. An example of serious respiratory tract infections could be seen through the frequent influenza epidemics and the deadly COVID-19 pandemic. All these epidemics and pandemics became a major public health burden worldwide. It was clear from various published literatures that there were three transmission routes of concern for respiratory viruses which were through droplet, airborne, and contact, including fomites [1,2,3]. Since all these viruses were transmitted through the respiratory route, their spread can likely be prevented using similar methods [4]. Over time, humans have developed various defence strategies against such viruses, including wearing face masks, implementing household quarantines, developing medicines, and creating vaccines that boost immunity against these pathogens. The use of non-drug interventions, such as wearing respiratory personal protective equipment (RPPE), was vital for protecting and decreasing the associated hazard of health care workers (HCWs) against respiratory infections when specialised vaccines or disinfection treatments are not available.

In view of the recent pandemic, the World Health Organization (WHO) and Centres for Disease Control and Prevention (CDC) recommended the public to wear face masks as part of the personal protective measures against the latest respiratory viral infection, COVID-19. Generally, there are 3 types of face masks being used which were

respirators, surgical and cloth face masks. Respirators, also known as the N95 face masks as well as the surgical face masks were widely used among the healthcare workers (HCW) while the cloth face masks were popular among the general population. Face masks were being used as a source control to prevent the respiratory virus from spreading through airborne droplets. Moreover, face masks were being used to protect the wearers by filtering the inhaled air from respiratory virus and bacteria. A systematic and meta-analysis on the effectiveness of cloth masks against respiratory viral infection has shown that cloth masks offer minimum efficacy and are inferior to N95 and surgical masks [5].

This review offered an essential and timely assessment of face mask usage and protection against respiratory tract infections. This review aimed to offer a comprehensive overview of the evidence showcasing the effectiveness of different types of face masks in preventing respiratory tract infections, both in community and healthcare settings. This review explored the following research question: Are face masks effective in preventing the transmission of respiratory tract infection in the general population?

As long as respiratory tract infections have not been eradicated, this review remains relevant for future use in upcoming endemic or pandemic outbreaks. Consequently, it was in our interest to develop a rapid review on the efficacy of face masks in deterring the transmission of respiratory tract infections.

## Materials and methods

A rapid review was conducted in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses [6]. A comprehensive searching strategy was meticulously used to select eligible studies from multiple electronic databases such as Cochrane Library, PubMed, ScienceDirect, HealthEvidence

and Google Scholar. Searches conducted on Google Scholar were mainly done to search for Gray literature related to the title of this rapid review. The following search terms were used for this rapid review: (“mask” OR “face mask” OR “effectiveness of face mask” OR “N95” OR “surgical mask”) AND (“respiratory tract infection transmission” OR “respiratory tract infection” OR “infection transmission”). No filters related to date or publication status were applied. Moreover, references from all the eligible articles were further searched to retrieve even more additional eligible studies. Articles that were not complete, only had abstracts and which were not related were all excluded. After eliminating duplicate entries, each abstract and title was critically reviewed by all three reviewers to filter out unrelated entries. In order to include relevant reviews, we obtained and studied the full text of any prospective research that met the selection criteria. The outcomes were analysed and discussed by all reviewers. If a discussion between any two reviewers failed to produce an agreement, the third reviewer was asked to reach a consensus. The extracted data were population types, the type of face mask used in the study, the type of setting, the effectiveness of face masks and the rate of infection transmission with and without face masks. Finally, the extracted data were reviewed for authenticity, accuracy, and completeness.

## Results

### Study selection

Six thousand four hundred forty-two (6442) articles were identified in the initial database search, of which 2479 articles had been removed as they were duplicates. The articles were screened based on their titles and abstract and 134 articles were retrieved. After excluding 111 articles for not meeting the inclusion criteria (reasons explained in Figure 1), 23 articles were eligible to be included in the study. Of the 23 studies, 11 were systematic reviews and meta-analysis, 3 were cluster randomised controlled

trials, 2 were literature review, 1 was cross sectional study, 1 was systematised review, 1 was pre-systematic review and 4 of the remaining studies were rapid reviews including 1 technical review.

Figure 1 below showed the study selection process of reports to review the effectiveness of face masks to prevent the transmission of respiratory tract infections (n=6442 studies).

### Study characteristics

Figure 2 represents the settings of the study conducted in the various papers. All of the studies (n=23) included in this review described the use of different types of face masks to prevent the transmission of respiratory tract infections. Table 1 summarises the types of masks used in the studies analysed. Figure 3 depicts the different types of face masks and corresponding number of studies that used them.

For the study population, 8 studies focused the efficacy of usage of face masks among healthcare workers. In one of the studies, it focused on the healthcare workers that were from the emergency departments and respiratory wards. 12 studies focused the effectiveness of face masks in general population, whereas one study focused on non-healthcare workers. There were a few studies that used specific area of population, one study used Asian population meanwhile another study focused on the Western population. One study used focused population of Hajj pilgrims. One study used volunteers with influenza-like illness, whereas one study focused on asymptomatic individuals without COVID-19 infection and confirmed COVID-19 patients. Majority of the studies (n =23) included in the review reported the use of various types of face masks to control the transmission of respiratory infections. Table 3 summarised the background of the studies together with the types of masks used to control the various respiratory infections and their effectiveness in preventing the transmission of respiratory tract infection.

## Discussion

The results regarding the effectiveness of face masks in preventing the transmission of respiratory infections were conflicting. A few studies ( $n = 3$ ) suggested that N95 respirators were more protective as compared to other types of face masks, including surgical or medical masks and cloth masks. According to a cluster randomized clinical trial (RCT) of 1441 hospital HCWs, N95 respirators were shown to be much less susceptible to bacterial colonisation, co-colonization, and viral-bacterial co-infection. Medical masks, on the other hand, were proven to be ineffective and may even increase the risk of viral co-infections. This result might be linked to the physical characteristics of medical masks that enhance moisture or other factors that raise the likelihood of co-infection [7]. The results correspond to those of a randomized clinical study that compared the efficacy of medical masks and N95 respirators (fit checked and non-fit checked) among HCWs. In the medical mask group, the incidence of infections such as clinical respiratory disease, influenza-like illness, laboratory-confirmed respiratory virus, and influenza was twice as high as in the N95 group [9]. Additionally, a literature review demonstrated that N95 respirators decreased substantially the probability of bacterial colonisation by 62% when compared to no mask and by 46% when compared to medical masks [11].

However, according to only one study ( $n = 1$ ) it was still uncertain if N95 respirators provide considerably greater protection than surgical masks for all patient care procedures in a healthcare setting [19].

While some of the studies concluded N95 was superior to surgical face masks, a few studies ( $n = 2$ ) involving N95 respirators and surgical masks suggest both masks were effective in reducing the risk of respiratory tract infections, SARS, and H1N1 among HCWs [16] as well as COVID-19 among the general population [18].

Additionally, few studies ( $n=5$ ) found that face masks in general were effective in mitigating respiratory infections. Systematic reviews and meta-analysis demonstrated that the use of face masks does provide protection against respiratory viral infections in general [20], decrease the risk of contracting SARS-CoV-2 infection [27], block or filter airborne virus-carrying particles [17] and could prevent spread of virus [14]. Furthermore, the length and severity of respiratory tract infection symptoms might be minimised when wearing masks [29]. A study conducted in both Asian countries and Western countries also discovered that wearing masks provided protective effects among both HCWs and non-HCWs [28].

Moreover, several studies ( $n = 5$ ) conducted to find the efficacy of surgical or medical masks revealed both significant ( $n = 4$ ) and non-significant ( $n=1$ ) protective effects against respiratory tract infections. A rapid review and meta-analysis demonstrated that medical face masks had a considerable protective impact in preventing the transmission of all respiratory tract infections, but this was contingent on compliance and use in conjunction with other preventative measures such as thorough hand hygiene [23]. Additionally, a systematic review of the effectiveness of surgical face masks against respiratory infections in mass gatherings, including among Hajj pilgrims and the HCWs who worked there, found significant protection against respiratory illnesses [26]. Furthermore, a study of a cluster RCT to explore the effectiveness, acceptability, and tolerability of non-pharmaceutical intervention in households with an influenza index patient reported statistically significant findings of decreasing influenza transmission across households in both the Mask group and the Mask with Hygiene group. This demonstrated that when implemented early and carefully, non-pharmaceutical interventions (NPI) such as face masks and increased hand hygiene could minimise household influenza transmission [25]. Next, a cross sectional study with voluntary sampling method investigated the

effectiveness of surgical face masks as a source control against Influenza A and Influenza B showed surgical face masks produced a 3.4-fold reduction in viral aerosol shedding. It was crucial to highlight that the number of viral copies in fine particle aerosols, as well as evidence for their infectiousness, implies that they play a key role in seasonal influenza transmission [24]. Besides, according to the findings of a systematic review and meta-analysis, using face masks might significantly decrease the clinical symptoms of respiratory infection in community settings [22]. Furthermore, findings from a systematic review conducted in non-healthcare settings, surgical masks had a moderate but non-significant protective impact on the frequency of acute respiratory infections (ARIs) [21].

In contrast, a few studies (n = 6) found no significant effectiveness of face masks in preventing respiratory infections. A systematic review assessing the efficacy of face masks against COVID-19 in healthcare settings discovered minimal data to support the effectiveness. However, the use of N95 respirators or air supplying respirators, as well as attention to personal hygiene guidelines, regular hand washing, and the use of disinfectants, could help to lower the prevalence of COVID-19 among healthcare personnel [8]. Next, a systematic review and meta-analysis to assess the effectiveness of face mask usage alone or in conjunction with hand hygiene in community settings in minimising the transmission of viral respiratory infection, found no significant decrease of ILI with the use of face masks, with or without hand hygiene, in these settings [10]. Furthermore, findings from a systematic review conducted in non-healthcare settings, indicated that surgical masks had a moderate but non-significant protective impact on the frequency of acute respiratory infections (ARIs) [21]. Similarly, a systematic review and meta-analysis found a non-significant protective effect of mask use in preventing influenza H1N1 infection. The findings indicated that campaigns promoting frequent hand hygiene, along with the use of face

masks in high-risk exposure situations, were likely to contribute to the prevention of pandemic influenza infection [15]. Additionally, there was low to moderate evidence from observational studies suggested that HCWs using face masks and PPE may be effective against COVID-19 [12]. However, another study found that both surgical and cotton masks might not efficiently filter SARS-CoV-2 from the environment or the exterior mask surface [13].

This rapid review has some limitations. First, there is still a lack of high-quality prospective studies with good design and research on mask use in the general population. Secondly, the mode of transmission for each respiratory infectious diseases influences the use, type and efficacy of masks; however, pertinent studies are very few. Thirdly, most of the studies did not address how long the masks were worn or whether they were worn correctly, which plays a huge role in determining how exactly the infection was transmitted. Finally, knowledge of additional influencing variables, including age, gender, culture, hand hygiene, and vaccination, might influence the protective effect of masks and thus needs to be explored in greater detail.

The results of this research, encompassing diverse study designs and exploring the effectiveness of different types of face masks across various groups and scenarios in preventing respiratory infections, revealed that well-fitted face masks were effective when worn consistently. To enhance infection prevention further, it is recommended that individuals also adopt other Non-Pharmaceutical Interventions (NPIs) like practicing good hand hygiene and maintaining social distancing.

## Conclusion

This rapid review highlighted the effectiveness of the usage of face mask in preventing the transmission of respiratory tract infections regardless of the type of setting, population, or respiratory pathogen. The findings clearly showed that the usage of masks does indeed offer

a certain degree of protection to both the individual and those around them.

Therefore, the policymakers should encourage face mask use among the general population for health protection. However, more evidence is required in terms of clarifying the usage of the mask in various settings and against diverse types of infection. Several reviews have highlighted the need for further research on personal protective equipment. This is crucial for drawing clearer conclusions and aiding higher authorities in making informed decisions on managing and controlling future pandemics or endemics effectively. It is challenging to prevent the spread of infectious diseases that are transmitted through droplet or airborne routes. In this era of newly emerging infectious diseases, infection prevention and control practices require continuous critical assessment. The public should be encouraged to wear face masks for health protection given the effectiveness of face mask use as a strategy for preventing respiratory infections. This review also demonstrate that there are geographical variations in how people perceive, intend to use face masks, and actually do so. These variations likely result from the diverse effects of various infectious diseases, regional cultures, and local legislation.. Authorities and allied organizations should work to reduce obstacles to wearing face masks and improve adherence by addressing stigma and bias

associated with mask use and educating the public through media and other communication channels.

#### **Conflict of interest**

The authors verified that there were no financial or commercial ties that might be viewed as having a potential conflict of interest.

#### **Declaration of competing interest**

None.

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#### **Authors contribution**

The first four authors designed, managed and wrote the paper as main contributors of this study. The rest of the authors provided intellectual and technical input to the manuscript for publication purpose.

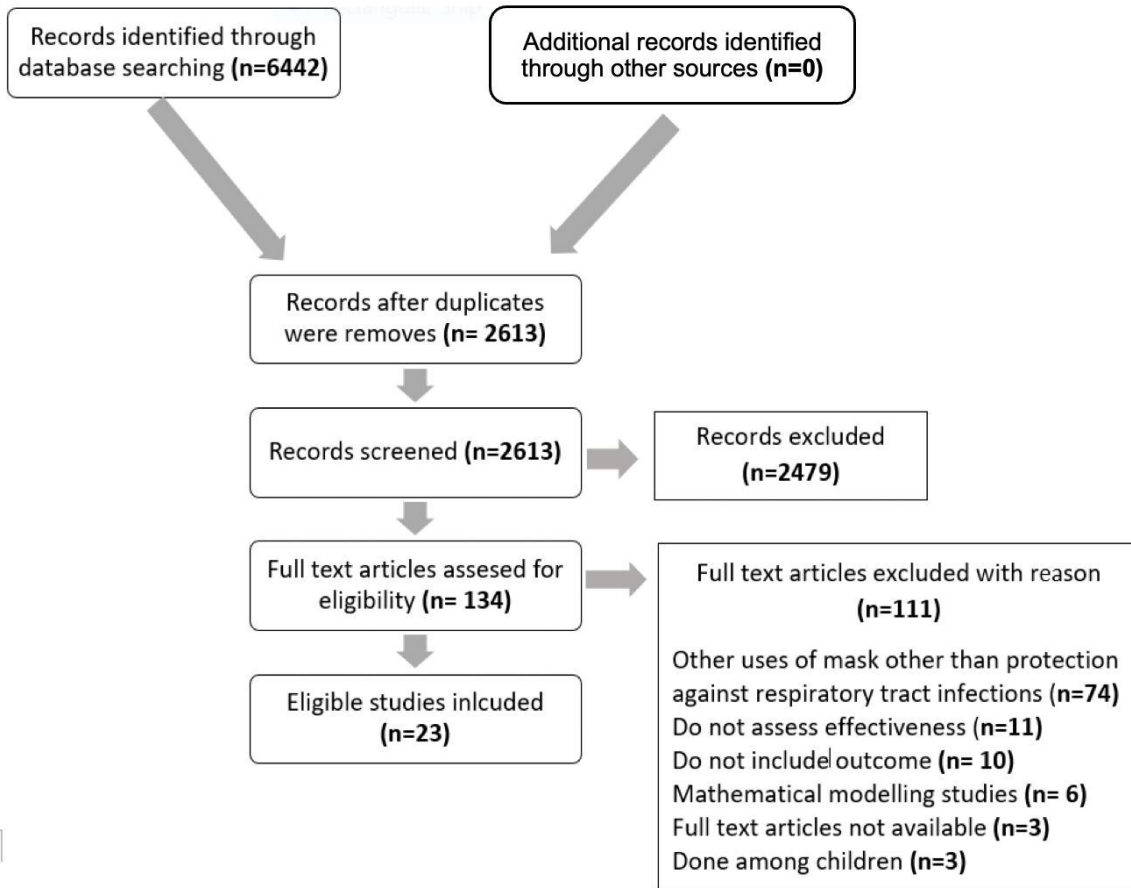


Figure 1. PRISMA flow diagram

Table 1. Types of face mask used and corresponding authors and number of studies

Type of face mask used	Authors	Number of studies (n=)
N95	MacIntyre, et al., 2014 (7), Dehaghi, Ghodrati-Torbati, Teimori, Ghavamabadi, & Jamshidnezhad, 2020 (8), MacIntyre, et al., 2011 (9), Aggarwal, Dwarakanathan, Gautam, & Ray, 2020 (10), MacIntyre & Chughtai, 2015 (11), Mohammad Ibrahim Khalil, 2021 (12), Pires, 2021 (13), Shaterian, Abdi, Kashani, Shaterian, & Darvishmotevalli, 2021 (14), Saunders-Hastings, Crispo, Sikora, & Krewski, 2017 (15), Offeddu, Yung, Low, & Tam, 2017 (16), Mingrui Liao, et al., 2021 (17), Abboah-Offei, et al., 2021 (18), Gamage, et al., 2005 (19) and Kim, et al., 2022) (20)	14
Surgical	Gamage, et al., 2005 (19), Wang, Gwee, Chua, & Pang, 2020 (21), Abboah-Offei, et al., 2021 (18), Mingrui Liao, et al., 2021 (17), Offeddu, Yung, Low, & Tam, 2017 (16), Saunders-Hastings, Crispo, Sikora, & Krewski, 2017 (15), Hui Li, et al., 2022 (22), Mohammad Ibrahim Khalil, 2021 (12), Pires, 2021 (13), Shaterian, Abdi, Kashani, Shaterian, & Darvishmotevalli, 2021 (14), Aggarwal, Dwarakanathan, Gautam, & Ray, 2020 (10), Kim, et al., 2022 (20), Chaabna, Doraiswamy, Mamtani, & Cheema, 2021 (23), MacIntyre & Chughtai, 2015 (11), MacIntyre, et al., 2011 (9), Milton, Fabian, Cowling, Grantham, & McDevitt, 2013 (24), Suess, et al., 2012 (25), Barasheed, et al., 2016 (26), MacIntyre, et al., 2014 (7) and Dehaghi, Ghodrati-Torbati, Teimori, Ghavamabadi, & Jamshidnezhad, 2020 (8)	19
Cloth	Mingrui Liao, et al., 2021 (17), Chaabna, Doraiswamy, Mamtani, & Cheema, 2021 (23) and Dehaghi, Ghodrati-Torbati, Teimori, Ghavamabadi, & Jamshidnezhad, 2020 (8)	3
Non-medical	Shaterian, Abdi, Kashani, Shaterian, & Darvishmotevalli, 2021 (14), Mohammad Ibrahim Khalil, 2021 (12), Pires, 2021 (13) and Kim, et al., 2022 (20)	4
Unspecified	Tabatabaeizadeh, 2021 (27), Liang, et al., 2020 (28), Brainard, Jones, Lake, Hooper, & Hunter, 2020 (29)	3



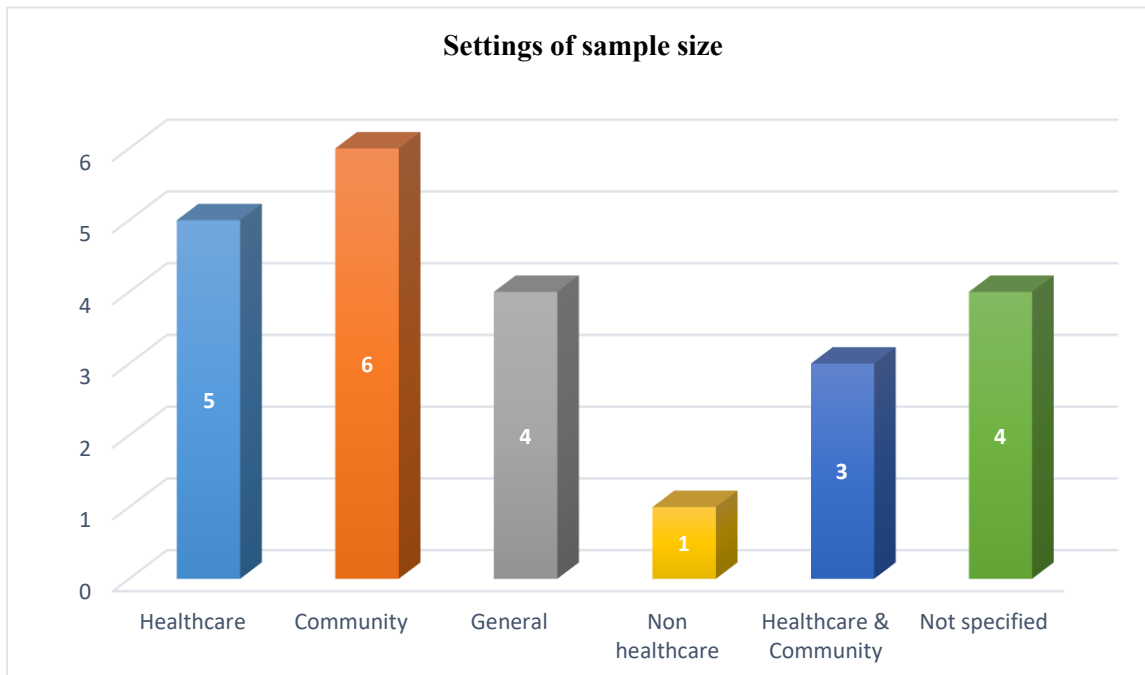


Figure 2. Settings of sample size

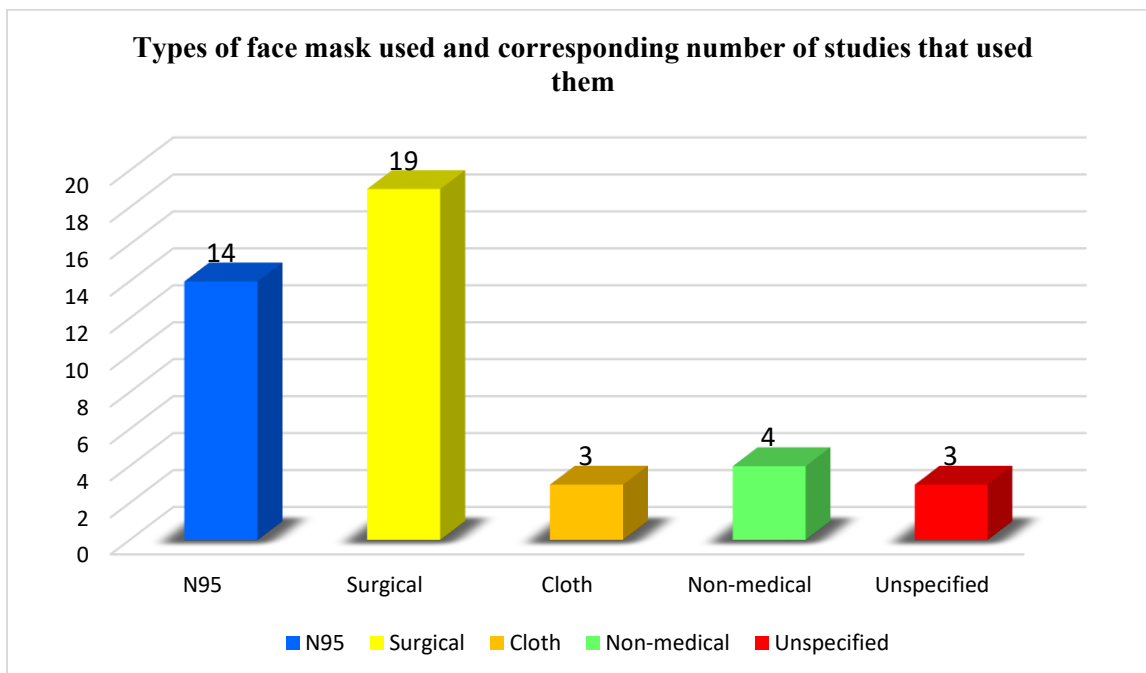


Figure 3. Types of face mask used and corresponding number of studies that used them

Table 1. Study population and corresponding authors

Authors	Study population
<p>Mohammad Ibrahim Khalil, 2021 (12), Dehaghi, Ghodrati-Torbati, Teimori, Ghavamabadi, &amp; Jamshidnezhad, 2020 (8), Liang, et al., 2020 (28), Offeddu, Yung, Low, &amp; Tam, 2017 (16), Barasheed, et al., 2016 (26), MacIntyre, et al., 2014 (7), MacIntyre, et al., 2011 (9) and Gamage, et al., 2005 (19)</p>	<p>Healthcare workers</p>
<p>Suess, et al., 2012 (25), MacIntyre &amp; Chughtai, 2015 (11), Aggarwal, Dwarakanathan, Gautam, &amp; Ray, 2020 (10), Chaabna, Doraiswamy, Mamtani, &amp; Cheema, 2021 (23), Kim, et al., 2022 (20), Pires, 2021 (13), Shaterian, Abdi, Kashani, Shaterian, &amp; Darvishmotevalli, 2021 (14), Hui Li, et al., 2022 (22), Mingrui Liao, et al., 2021 (17), Abboah-Offei, et al., 2021 (18), Wang, Gwee, Chua, &amp; Pang, 2020 (21) and Brainard, Jones, Lake, Hooper, &amp; Hunter, 2020 (29)</p>	<p>General population</p>
<p>Liang, et al., 2020 (28)</p>	<p>Non healthcare workers, Asian population, Western population</p>
<p>Barasheed, et al., 2016 (26)</p>	<p>Pilgrims</p>
<p>Milton, Fabian, Cowling, Grantham, &amp; McDevitt, 2013 (24)</p>	<p>Volunteers with influenza-like illness from the Lowell, MA community, primarily among students and staff of the University of Massachusetts</p>
<p>Saunders-Hastings, Crispo, Sikora, &amp; Krewski, 2017 (15)</p>	<p>Humans exposed to a pandemic influenza</p>

Table 3. Studies included on face mask use in preventing respiratory tract infections

No	Author / Year / Country	Study aim	Population	Sample size	Settings	Types of masks	Type of respiratory infection	Study design	Effectiveness of face mask	Conclusion/Remarks
1.	MacIntyre, et al., 2014 (7) Beijing, China	To compare the efficacy of medical masks and N95 respirators in preventing bacterial colonization/infection in healthcare workers (HCWs).	HCW: nurses and doctors	1922 participants (1441 randomized and 481 control)	Health care	N95 and surgical face masks	Respiratory bacterial infections: Streptococcus pneumoniae, Bordetella pertussis, Chlamydia pneumoniae, Mycoplasma pneumoniae or Haemophilus influenzae type B	Cluster randomized controlled trial	59% efficacy against control of N95 respirators against any co-infection, and 67% against bacterial/viral co-infection. Medical masks were not protective and may in fact increase the risk of viral co-infections.	It is possible that the physical conditions of a medical mask may increase moisture or other parameters to increase risk of co-infection.
2.	Dehaghi, Ghodrati-Torbati, Teimori, Ghavamabadi, & Jamshidnezhad, 2020 (8) Seoul, Wuhan,	To assess the effectiveness of face masks against the novel coronavirus.	HCW	5 studies	Health care	N95, surgical face masks and cloth face masks	COVID-19	Systematic review	There is little evidence to support the effectiveness of face masks to reduce the risk of COVID-19 infection. However, the use of N95	More studies in controlled contexts and studies of infections in healthcare and community places are needed for better definition of the

	Hong Kong								respirators or air supplying respirators and adherence to the principles of personal hygiene, frequent hand washing, and the use of disinfectants can reduce the prevalence of COVID-19 in healthcare providers.	effectiveness of face masks in preventing coronavirus.
3.	Barasheed, et al., 2016 (26) 55 countries	To synthesise evidence about the uptake and effectiveness of face mask against respiratory infections in mass gatherings.	HCW and Hajj pilgrims	25 studies	Health care and community	Surgical face masks	Respiratory infections	Systematic review	Only 13 studies examined the effectiveness of face mask, and their pooled estimate revealed significant protectiveness against respiratory infections (relative risk [RR]=0.89, 95% CI: 0.84-0.94, p<0.01), but the study end points varied widely.	The main limitation is that most of the studies were of 'average' or 'below average' quality.
4.	Suess, et al., 2012 (25)	To investigate efficacy, acceptability	General population	302 participants	Community	Surgical face masks	Influenza H1N1, Influenza	Cluster randomised	Statistically significant results of lowered	Household transmission of influenza can be

	Berlin, Germany	y, and tolerability of non-pharmaceutical intervention in households with influenza index patient.	which involving households above 14 years old				a B and Influenza-like illness	controlled trial	influenza transmission among households in both Mask group and Mask with Hygiene group.	reduced using non-pharmaceutical interventions (NPI), such as face masks and intensified hand hygiene, when implemented early and used diligently.
5.	Milton, Fabian, Cowling, Grantham, & McDevitt, 2013 (24) USA	To investigate the effectiveness surgical face masks as a source control.	Volunteers with influenza-like illness from the Lowell, MA community, primarily among students and staff of the University of Massachusetts	89 participants	Community	Surgical face masks	Influenza A and Influenza B	Cross sectional study design with voluntary sampling method	Overall, surgical face masks produced a 3.4-fold reduction in viral aerosol shedding.	The abundance of viral copies in fine particle aerosols and evidence for their infectiousness suggests an important role in seasonal influenza transmission.
6.	MacIntyre, et al., 2011 (9) Beijing, China	To compare the efficacy of medical masks, N95 respirators	Hospital HCWs aged above 18 years old	1441 participants	Health care	N95 respirators fit-tested, N95 respirators	Clinical respiratory illness, Influenza-like illness, laboratory	Cluster randomized clinical trial	The rates of CRI (3.9% versus 6.7%), ILI (0.3% versus 0.6%), laboratory-confirmed respiratory	Rates of infection in the medical mask group were double that in the N95 group. A benefit of

		(fit tested and non-fit tested), in HCW.	and from the emergency departments and respiratory wards of 15 hospitals.			non-fit-tested and medical masks	ry-confirmed viral respiratory infection and laboratory-confirmed influenza A or B.		virus (1.4% versus 2.6%) and influenza (0.3% versus 1%) infection were consistently lower for the N95 group compared to medical masks.	respirators is suggested but would need to be confirmed by a larger trial, as this study may have been underpowered.
7.	MacIntyre & Chughtai, 2015 (11)	To inform policy makers and stakeholders by examining and summarising the available evidence related to the efficacy of face masks and respirators, current practice, and guidelines, as well as highlighting the gaps in evidence.	General population	14 studies	Community and healthcare	Medical masks and N95 respirators	Respiratory infection (Viral infection, bacterial infection, influenza)	Literature review	None of the four RCTs showed that medical masks were efficacious, although efficacy might have been at a lower level than the trials were able to detect. N95 respirators significantly reduced the risk of bacterial colonisation by 62% compared with no mask and by 46% compared with medical masks, which were not efficacious.	Health economic analyses of face masks are scarce and the few published cost effectiveness models do not use clinical efficacy data. The lack of research on face masks and respirators is reflected in varied and sometimes conflicting policies and guidelines.

8.	Aggarwal, Dwarakanathan, Gautam, & Ray, 2020 (10)	To estimate the effectiveness of face mask, use alone or along with hand hygiene in community settings in reducing the transmission of viral respiratory illness	General population	17 studies	Community	Medical masks and N95 respirators	Influenza-like illness	Systemic review and Meta-analysis	The pooled estimate of the randomized control trials did not show any significant reduction of ILI using face masks with or without hand hygiene in community settings.	Available evidence does not confirm a protective effect of face mask usage alone in a community setting against influenza-like illnesses (and potentially, the COVID-19). For maximum benefit, mask use should be combined with other essential non-pharmaceutical interventions like hand hygiene.
9.	Chaabna, Doraiswamy, Mamtani, & Cheema, 2021 (23)	To inform policymakers and stakeholders by examining and synthesizing available evidence on the effectiveness of cloth and medical face masks for preventing transmission of respiratory	General population	12 primary studies	Community	Medical and cloth face masks	Influenza, influenza-like illness, SARS-CoV, and SARS-cov-2	Rapid review and Meta-analysis	The current meta-analysis demonstrated a significant protective effect of medical face mask use (combined or not with other interventions) in preventing the transmission of all respiratory infections, including	Medical face mask effectiveness is dependent on compliance and utilization in combination with preventive measures such as intensive hand hygiene.

		infections in community settings and pointing out the gaps in evidence.							SARS- CoV -2 and SARS- CoV	
10	Kim, et al., 2022 (20)	To evaluate the comparative prevention effectiveness of the most common types of face maskss (N95 respirators, surgical or medical masks, and non-medical masks) that have been used as personal protective equipment (PPE) by network meta-analysis	General population	35 articles	Health care and community	N95 respirators, surgical or medical masks, and non-medical masks	Respiratory viruses (influenza virus, SARS-CoV, MERS-CoV, and SARS-cov-2)	Systematic review and Meta-analysis	Our study demonstrated that the use of face masks provides protection against respiratory viral infections in general. Among various types of face masks, it is slightly safer to use N95 or equivalent in healthcare settings as PPE for the moment until more evidence on other types of masks are realised.	Our study confirmed that the use Of face masks provides protection against respiratory viral infections in general. However, the effectiveness may vary according to the type of face mask used. Our Findings encourage the use of N95 respirators or their equivalents for best Personal protection in healthcare settings until more evidence on surgical and



										Medical masks are accrued. This study highlights a substantial lack of evidence on the Comparative effectiveness of mask types in community settings.
11	Mohammad Ibrahim Khalil, 2021 (12)	To quantify the risk of COVID-19 among surgeons and explore whether face masks and other PPE could minimise the risk of COVID-19 among surgeons.	Health care workers	14 primary studies and 11 systematic reviews	Health care	N95 respirators, surgical or medical masks, and non-medical masks	SARS-cov-2	Systemised review (Not a systematic review)	There is low to moderate evidence from observational studies that face mask and PPE use by HCWs may be beneficial against COVID-19, although these are grossly underutilized.	We note that surgeons are at risk of COVID-19, although the precise risk could not be estimated.
12	Pires, 2021 (13)	To review, analyse and discuss all works about the wearing and development of face masks as potential protection	General population	21 studies	Not specified	N95 respirators, surgical or medical masks, and non-medical	SARS-CoV-2	Pre-systematic review	Worryingly, during coughing, both surgical and cotton masks may not effectively filter SARS-cov-2 from the environment	-

		against SARS-COV-2 during the COVID-19 pandemic.				1 masks			and external mask surface, which is also reinforced by the facts that particles 0.04 to 0.2 μm can penetrate surgical masks and that surgical masks and unvented KN95 respirators only reduce the outward particle emission rates by 90% and 74% (average values), respectively, when speaking and coughing in comparison to wearing no mask.	
13	Shaterian, Abdi, Kashani, Shaterian, & Darvish motevalli, 2021 (14)	Aimed to assess the impact of face masks and respirators on reducing the spread of respiratory viruses.	General population	10 articles	Not specified	N95 respirators, surgical or medical masks, and non-medical masks	SARS-CoV -2, MERS, MERS-CoV, Rhinovirus, Influenza A viruses (H1N1), Influenza B	Systematic review	This systematic review showed that using face masks or respirators aided in preventing the spread of respiratory viruses. The result of the present	We recommend conducting more studies on the effect of each type of face mask and respirator, individually, and on the prevention of the spread of different viruses.

							viruses, Parainfluenza 1,2 and 3 viruses, Enteroviruses, Adenoviruses, Human metapneumoviruses, Respiratory syncytial virus A or B, Coronaviruses, Picornaviruses, and Enteroviruses		systematic review showed that using face masks could prevent the spread of virus.	Moreover, we suggest assessing the effect of simultaneous use of masks, duration of using a face mask, and distance between healthy people and the person infected with respiratory viruses.
14	Hui Li, et al., 2022 (22)	This systematic review and meta-analysis aimed to firstly evaluate the efficacy of medical masks use on reducing the respiratory infection in community settings,	General population	8 RCT studies on efficacy of face masks use, 78 studies for perception, intention, and practice towards wearing face mask.	Community	Medical mask	Respiratory infection	Systematic Review and Meta-analysis	Our results suggest face mask use may significantly reduce the clinical symptoms of respiratory infection.	The governments and related organizations should make effort to increase the compliance of face mask use and reduce barriers associated with the use of face masks, such as reducing stigma and prejudice on face mask use,

		and secondly estimate the perception, intention, and practice regarding wearing face masks among the general population during infectious disease pandemics.								public education through media and other communication channels.
15	Saunders - Hastings, Crispo, Sikora, & Krewski, 2017 (15)	To examine the effectiveness of personal protective measures in preventing pandemic influenza transmission in human populations.	Humans exposed to a pandemic influenza	16 studies (Eight studies evaluated the effectiveness of face mask use in preventing pandemic influenza infection)	Not specified	Hand hygiene, surgical masks, and N95 respirators	H1N1 Influenza	Systematic Review and Meta-analysis	Meta-analysis found a non-significant protective effect of mask use in preventing influenza infection	Despite persisting knowledge gaps in relative effectiveness between interventions and across population groups, results suggest that campaigns to increase the frequency of hand hygiene, alongside use of face masks in situations with a high risk of exposure, are likely to contribute to preventing pandemic

										influenza infection.
16	Offeddu, Yung, Low, & Tam, 2017 (16)	To develop evidence-based recommendations to reduce the occupational risk of respiratory infection among medical personnel.	Health care workers	29 studies (23 observational studies and 6 randomized controlled trials)	Not specified	N95 respirators and medical mask	SARS H1N1	Systematic Review and Meta-analysis	We found evidence to support universal medical mask use in hospital settings as part of infection control measures to reduce the risk of respiratory tract infection among HCWs	Our analysis confirms the effectiveness of medical masks and respirators against SARS.
17	Brainard, Jones, Lake, Hooper, & Hunter, 2020 (29)	To assess effectiveness of wearing face masks in the community to prevent respiratory disease and recommend improvements to this evidence base.	General settings	33 studies (12 randomized control trials (RCTs) were included.	Community	Not specified	Respiratory illness, such as from coronaviruses, rhinoviruses, influenza viruses or tuberculosis	Rapid scoping review	It is possible that face mask wearing reduced duration or severity of symptoms experienced due to reducing infectious dose received, although not actual disease.	Wearing face masks may reduce primary respiratory infection risk, probably by 6-15%.
18	Liang, et al., 2020 (28)	To evaluate the effectiveness of the use of masks to	Health care workers, non-healthcare worker	21 studies	General	Not specified	Influenza, SARS, Covid-19	Systematic review and meta-analysis	Wearing masks does provide protective effects in both Asian countries and	This study adds additional evidence of the enhanced protective value of

		prevent laboratory-confirmed respiratory virus transmission.	s, Asian population, Western population						western countries by 69% and 55%, respectively. Among HCWs, it reduced the risk in both Asian and western countries. Especially, for non-healthcare populations, reduced risk of 54% was found in western countries, and a reduced risk of 49% was found in Asia.	masks, we stress that the use masks serve as an adjunctive method regarding the COVID-19 outbreak.
19	Mingrui Liao, et al., 2021 (17)	To review the design, manufacturing, functional performance, and effectiveness of various types of face masks.	General population	4 studies	General	N95 mask, surgical mask, and cloth mask	Covid-19	Technical review	Face masks wearing can block or filter airborne virus-carrying particles through the working of colloid and interface science.	Although the current demand has driven many commercial masks from various manufacturers and suppliers, there is a lack of agreed standards or requirements concerning their manufacturing and testing that would provide

										evidence-based guidelines on their safe use for the general public, and hence, fit for purpose.
20	Abboah-Offei, et al., 2021 (18)	To investigate the impact face mask use has had in controlling transmission of respiratory viral infections.	General population	58 studies (13 systematic reviews and 45 quantitative studies)	General	N95 and surgical mask	Covid-19	Rapid review	Face masks use have shown a great potential for preventing respiratory virus transmission including COVID-19.	Regardless of the type, setting, or who wears the face mask, it serves primarily a dual preventive purpose, protecting oneself from getting viral infection and protecting others. Therefore, if everyone wears a face mask in public, it offers a double barrier against COVID-19 transmission.
21	Wang, Gwee, Chua, & Pang, 2020 (21)	This review aims to summarize and assess the association between surgical mask wearing	General population	23,892 (15 studies)	Non healthcare	Surgical mask	Acute respiratory illness	Systematic review	A modest but non-significant protective effect of surgical masks on acute respiratory illnesses (ARIs)	Surgical mask wearing among individuals in non-healthcare settings is not significantly associated with reduction in ARIs

		and acute respiratory infection incidence, from existing interventional and observational studies conducted in non-healthcare settings.							incidence was observed.	incidence in this meta-review
22	Gamage, et al., 2005 (19)	The purpose of this paper is to review the findings of a literature search on the effectiveness of infection control practices aimed at preventing occupational-associated transmission of infectious respiratory agents in the health care setting	Health care workers	168 studies	Health care	Surgical mask and N95 respirators	SARS	Literature review	It is still unclear whether N95 respirators offered significantly better protection than surgical masks for all patient care activities	Failure to implement appropriate barrier precautions is responsible for most nosocomial transmissions
23	Tabatabaizadeh, 2021 (27)	This study is aimed to investigate the airborne	Asymptomatic individuals	7688 participants (4 Studies)	General	Not specified	Covid-19	Meta-analysis.	Meta-analysis of studies has suggested that the use	In conclusion, there is association between face mask use and



	transmission of COVID-19 and the role of face mask to prevent it.	without COVID-19 infection and confirmed COVID-19 patients.						of the face mask was associated with a decreased risk of SARS-CoV-2 infection ( $P < 0.001$ )	reduction of COVID-19
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## MINI REVIEW

# Can *Syzygium aromaticum* and *Eucalyptus globulus* Ease Respiratory Infection?

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### Abstract

In response to the growing demand for effective treatments for respiratory infections, research has focused on the therapeutic properties of natural substances like *Syzygium aromaticum* (clove) and *Eucalyptus globulus* (Eucalyptus). Clove is rich in phytochemicals such as eugenol, which offers antimicrobial, anti-inflammatory, and antioxidant benefits in managing respiratory infections. Similarly, Eucalyptus contains eucalyptol, which is known for its antimicrobial and anti-inflammatory properties. This review systematically examines research on the active constituents of clove and Eucalyptus through a detailed analysis of scientific literature from databases such as Web of Science, Scopus, Google Scholar, and Research Gate. By assessing the phytochemical and pharmacological properties of these plants, the review highlights their therapeutic potential in treating respiratory infections. It also emphasises the need to explore further their use in developing innovative treatment strategies for respiratory health.

**Keywords:** *Eucalyptus globulus*, pharmacological properties, phytochemical, respiratory infections, *Syzygium aromaticum*.

## Introduction

Respiratory infections (RIs) are infections that affect the respiratory system, including the nose, throat, airways, and lungs [1]. Clinically, RIs are classified into upper respiratory tract infections (URTIs), involving the nose, sinuses, pharynx, larynx, and trachea, and lower respiratory tract infections (LRTIs), which affect the airways and lungs below the larynx [2,3]. Common URTIs include the common cold, sinusitis, pharyngitis, laryngitis, and rhinitis, while LRTIs include pneumonia, bronchitis, and bronchiolitis [1–3]. These infections can be caused by viruses, bacteria, or fungi and they typically present with symptoms such as coughing, rhinorrhea, and nasal congestion [4,5].

Conventional methods to treat respiratory infections may involve using drugs that alleviate symptoms and eliminate the infection. Antibiotics are administered to cure bacterial infections when present, while other medications, such as bronchodilators, can aid in expectoration and relieve wheezing [4,6]. However, a drawback is that antibiotics have undesirable effects on bowel microflora and may lead to antibiotic resistance if used improperly [7,8]. At other times, respiratory illnesses are caused by viruses, so antibiotics will not be effective against them [5]. The prolonged use of antiviral drugs may also cause antiviral resistance [9]. The worldwide market for RIs treatment was valued at USD 33,534.13 million in 2021 [10]. According to research conducted by Business Research Insights, the RIs market is projected to escalate to a valuation of USD 53,207.21 million by 2027 [10]. This evidence underscores the necessity for interventions addressing RIs, encompassing traditional and complementary treatments.

In addition to conventional medicine, many individuals turn to herbal remedies for relief from respiratory infections [11,12]. Herbs have long been used in traditional medicine to treat respiratory conditions, which has prompted increased scientific interest in their therapeutic potential [12–14]. Plants such as *Syzygium aromaticum* (commonly known as clove) and *Eucalyptus globulus* (blue gum tree) contain

various phytochemicals and metabolites that can help alleviate RIs [16].

*S. aromaticum*, commonly known as clove, refers to the aromatic flower buds widely utilized as a spice in Asian, African, Mediterranean, and Middle Eastern cuisines [14]. It also has been a staple in traditional medicine systems such as Ayurveda and Chinese medicine for centuries [13]. The essential oil derived from *S. aromaticum* buds is abundant in phytochemicals such as eugenol,  $\alpha$  and  $\beta$ -caryophyllene,  $\alpha$ -copaene, and methyl-salicylate, which serves a variety of medicinal purposes, such as promoting oral health, relieving pain, and exhibiting antibacterial and anti-inflammatory properties [13,15]. In respiratory infection, *S. aromaticum* can function as an expectorant and antimicrobial agent to alleviate the symptoms [16,17].

*E. globulus*, also known as the blue gum tree, is an evergreen tree in the Myrtaceae family [11,18]. Its essential oil is obtained from the leaves through steam distillation and has been extensively researched for its diverse properties and applications. Studies indicate that the oil possesses antimicrobial properties that are effective against bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), serving as an expectorant to alleviate phlegm and relieve congestion [19,20]. Furthermore, eucalyptus oil, along with its constituent phytochemicals such as cineole,  $\alpha$ -pinene, limonene, globulol, and flavonoids, has been shown to exhibit anti-inflammatory properties [11,21,22]. These components contribute to mitigating inflammation within the respiratory tract [20,22]. Additionally, the oil exhibits antiviral and antifungal properties, making it beneficial in addressing a variety of respiratory infections [6,22].

Studies on these plants concerning respiratory health remain limited over the years. Most studies have focused on phytochemical analysis, production and its application in several products [12,13]. A document that collates the most important and relevant studies, highlighting the main trends and developments concerning these

plants' potential for respiratory health, is necessary. Therefore, this mini-review delves into the potential impact of *S. aromaticum* and *E. globulus* in improving RIs by analysing each of the plants most popular phytochemicals. Additionally, it scrutinizes the pharmacological attributes of these phytochemicals and their roles in ameliorating RIs.

## Methodology

This review examines the effects of *S. aromaticum* and *E. globulus* on improving RIs. It gathers information through a selection process, including research articles and narrative reviews that explore the plants' phytochemicals (natural chemicals) and pharmacological properties (drug-like effects). The inclusion criteria of this review consider studies conducted in both laboratory settings (*in vitro*) and on living organisms (*in vivo*) and focus on each plant's prominent phytochemicals and important pharmacological properties. Apart from that, non-English and retracted articles were excluded. Articles were systematically searched from various databases, including Web of Science, Scopus, Google Scholar, and Research Gate, to ensure a comprehensive analysis.

## Results and discussions

### Phytochemicals of *Syzygium aromaticum* and *Eucalyptus globulus*

*S. aromaticum* contains various phenolic compounds, including hydroxyphenyl propene, hydroxycinnamic acids, flavonoids, hydroxybenzoic acids, and eugenol, that improve RIs [15]. These compounds constitute the most prevalent bioactive components in the fresh plant and its essential oil, with a total phenolic content of around 9.07 gallic acid equivalent (GAE) mg/g [23]. Table 1 presents the major compositions and properties of clove.

Amongst the previously mentioned components of *S. aromaticum*, eugenol,  $\alpha$ - and  $\beta$ -caryophyllene, and eugenol acetate are the

primary and most prevalent phenolic compounds in fresh plant and essential oil [15, 24]. Eugenol has several pharmacological properties, including antioxidant capacity, antibacterial activity, neuroprotective ability, hypolipidemic efficiency, anti-inflammatory action, anti-carcinogenic effects, anti-diabetic effectiveness and therapeutic potential against respiratory distress [25]. Caryophyllene is soluble in ethanol but not in water. It has demonstrated local anaesthetic effects and antibacterial, anxiolytic, antioxidant, anti-inflammatory, and anticancer activities, including effectiveness against breast, cervical, prostate, and pancreatic cancers [15, 26]. Eugenyl acetate, a eugenol derivative, also showed antibacterial, anticancer, antioxidant, and antiviral properties [15].

Apart from that, compounds like chlorogenic acid, caffeic acid, gallic acid and p-coumaric acid from the phenolic acid group; kaempferol, myricetin, rhamnetin, quercetin and epicatechin from the flavonoids group; cyanidin compound from anthocyanin group, tannin also can be found in *S. aromaticum* as shown in Table 1.

*E. globulus* contains abundant phytochemicals, such as monoterpenes, oxygenated monoterpenes, sesquiterpenes, and triterpenic acids, which may help improve RIs (Table 1) [27–28]. These phytochemicals are commonly isolated from the leaves and have demonstrated broad-spectrum antiviral, antibacterial, anti-inflammatory, and antioxidant properties [11,19,22,29]. Prominent phytochemicals in *E. globulus* that aid in improving RIs are eucalyptol,  $\alpha$ -pinene, limonene, globulol, and p-cymene [27]. Eucalyptol, also known as 1,8-Cineole, is a key component in *E. globulus* that helps to reduce RIs. This natural monoterpene (bicyclic ether) compound makes up 60-80% of *E. globulus* composition and primarily derives from the plant's leaves [22,30]. Eucalyptol is known for its antimicrobial, mucolytic and spasmolytic effects on the respiratory tract and is effective in treating respiratory infections [11,19]. It also demonstrates anti-inflammatory and antioxidant

properties, enhancing its therapeutic benefits in managing RIs [31,32].

The second most prevalent phytochemical found in *E. globulus* is  $\alpha$ -pinene, typically comprising 7-11% of the total compounds in the plant [27,28].  $\alpha$ -pinene is a bicyclic monoterpene with a structure consisting of a six-membered ring fused to a four-membered ring and a double bond within the six-membered ring [20,32].  $\alpha$ -pinene has demonstrated therapeutic properties against upper respiratory tract infections due to its antimicrobial properties [11]. This compound also possesses anti-inflammatory properties that can soothe RIs [31].

Limonene is another intriguing component found in *E. globulus*, although it is present in smaller amounts (around 7%) compared to eucalyptol and  $\alpha$ -pinene [27,28]. Limonene is a monocyclic monoterpene with a structure similar to  $\alpha$ -pinene. The only difference is the number of cyclic structures;  $\alpha$ -pinene has two bicyclic structures [20,32]. Limonene plays a crucial role in the plant's defense mechanisms and contributes to the biological activities of *E. globulus* [17]. However, research has indicated that limonene possesses anti-inflammatory and antioxidant properties, contributing to its therapeutic effects in respiratory infections [11,21].

p-Cymene, known as 1-isopropyl-4-methylbenzene, is another monoterpene compound that can be extracted from *E. globulus* leaves. p-Cymene typically comprises about 7% of the total *E. globulus* composition and shows promise as a therapeutic agent for managing respiratory infections due to its anti-inflammatory, antioxidant, antimicrobial, and analgesic properties [20,27,32]. Globulol is a bicyclic sesquiterpene alcohol that has been extracted from *E. globulus*. Around 6% of globulol can be found in *E. globulus* compositions [28]. Although globulol is a minor component in *E. globulus*, it has demonstrated strong antioxidant and antimicrobial properties, which could aid in reducing respiratory infections [28,31]. Other compounds that may help reduce RIs are  $\beta$ -pinene,  $\beta$ -myrcene and  $\gamma$ -terpinene (Table 1).

### **Pharmacological properties of *Syzygium aromaticum* and *Eucalyptus globulus***

Traditional medicine has used *S. aromaticum* as a respiratory, commonly used to address respiratory ailments such as coughs, colds, asthma, bronchitis, and sinusitis [33,34]. One method involves inhaling the aroma of hot clove tea during an aromatherapy session. In Asian cultures, it is customary to chew cloves to alleviate throat soreness and pharynx inflammation [33,34]. Additionally, chewing cloves after they have been heated is known to provide relief from intense coughing. *S. aromaticum* oil is also recognised for its expectorant, antiviral and antimicrobial properties, making it effective in treating respiratory disorders like colds, bronchitis, coughs, asthma, and other upper respiratory conditions [15,35]. On top of that, the active compound eugenol in *S. aromaticum* oil has been shown to disrupt the cellular membranes of bacteria like *Salmonella* and *Helicobacter pylori* [36,37].

Despite the many properties of *S. aromaticum*, studies on the plant's respiratory health are limited. A recent study by Chniguir et al. investigated the antioxidant potential of the plant aqueous extract and its protective effects on lipopolysaccharide (LPS)-induced lung in mice [38]. The study found that the plant inhibited the activity of myeloperoxidase, an enzyme in human neutrophils that can contribute to inflammation and oxidative stress. Specifically, the researchers showed that *S. aromaticum* could inhibit the generation of reactive oxygen species, superoxide anion, and hydrogen peroxide by human neutrophils. Furthermore, the study demonstrated that *S. aromaticum* had anti-inflammatory effects in a mouse model of LPS-induced lung inflammation. When *S. aromaticum* was administered intraperitoneally to mice before LPS exposure, it reduced the influx of inflammatory cells and the total protein content in the bronchoalveolar lavage fluid, indicating a protective effect against LPS-induced lung inflammation. Based on the study, the antioxidant

and anti-inflammatory properties of *S. aromaticum* make it a promising candidate for developing new therapeutic approaches to treat lung inflammation and related respiratory disorders [38].

The traditional medicinal application of *S. aromaticum* for respiratory illnesses documented antiviral effects against various viruses, and their anti-inflammatory and antioxidant properties collectively underscore the potential significance of *S. aromaticum* and its phytochemical components in respiratory problems.

Various studies have been conducted to evaluate *E. globulus* efficiency in improving RIs. One study examined eucalyptol, a major component of *E. globulus*, to investigate its effects on respiratory tract immunity and overall immune function in rat models [20]. This study aimed to investigate the impact of eucalyptol on the respiratory and immune function of CD8 and CD4 cells, as well as alveolar macrophages. The findings indicated that low and moderate doses (30 and 100 mg/kg) of eucalyptol positively affected CD8+ T cells, enhancing respiratory immune function [20]. However, high doses (300 mg/kg) had an inhibitory effect and impaired overall immune function [20]. These results demonstrate that eucalyptol can strengthen the immune system in the respiratory tract, potentially reducing the risk of respiratory infections. However, it is important to note that dosage plays a crucial role, as high doses can have detrimental effects on the respiratory tract.

Several studies have been conducted to confirm the strong antimicrobial properties of *E. globulus*. Salari et al. investigated the impact of *E. globulus* on pathogenic bacteria isolated from patients with respiratory tract infections [29]. Their findings showed that *E. globulus* was able to kill the common respiratory pathogens, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* at concentrations of 512, 128, 64, and 64 mg/L, respectively [29]. Another study explored the antiviral properties of both *E. globulus* oil and its primary active compound,

eucalyptol [39]. The results have demonstrated *in vitro* antiviral activity against the respiratory virus influenza A (H1N1) [39]. It was found that the *E. globulus* involves the inactivation of free virus particles and disruption of the viral envelope structure.

A study of late rhinosinusitis using a human *ex vivo* model mimicking the human nose found that eucalyptol effectively reduced mucus hypersecretion [30]. The research involved treating nasal slice cultures with lipopolysaccharides to simulate bacterial infection in late rhinosinusitis, significantly increasing mucin-filled goblet cells. However, the group treated with eucalyptol showed a significant reduction in the number of mucin-filled goblet cells compared to the lipopolysaccharide-treated group [30]. At a molecular level, eucalyptol significantly decreased the expression levels of the mucin genes MUC2 and MUC19, associated with reduced NF-κB activity [30].

In a research study that investigated the impact of eucalyptus inhalation on upper respiratory tract infection (URTI) in 208 children aged 5-15 years [40], the participants were treated by inhaling 2 drops of eucalyptus oil mixed in 750 mL of hot water every 3 hours for 15 minutes over 3 days. The control group only received water vapour. The study revealed that eucalyptus fumigation notably relieved symptoms associated with various upper respiratory infections (URIs), such as colds, rhinitis, sinusitis, pharyngitis, otitis, and laryngotracheitis compared to the control group [40]. Table 2 shows more *in-vitro*, *in-vivo* and clinical trial studies involving *S. aromaticum* and *E. globulus* in treating RIs.

### **Limitations and future directions**

Several limitations and gaps must be addressed despite *S. aromaticum* and *E. globulus* showing promising results in improving RIs. The current studies showed that varying protocols and methodologies for scientific research and clinical studies, such as actions in dosage variation, formulations, and delivery systems, create



challenges in drawing consistent conclusions about their efficacy. Additionally, some studies may point to potential drug interactions when these two plants were administered to the patients, there is also a need for more research addressing these plants' interactions with drugs, toxicity and side effects, particularly in diverse populations. To overcome these limitations, future studies should prioritise conducting standardised clinical trials, mainly focusing on the formulations, dosage, and delivery systems, to assess the effectiveness and safety of *S. aromaticum* and *E. globulus* in treating respiratory infections. Additionally, it would be beneficial to explore the underlying mechanisms of action of the active compounds in these plants, especially their molecular mechanisms. Drug interaction studies can also be intensified to identify any adverse effects or contraindications of these plants with the commercialised drugs in the market. More toxicity assessments, including *in-vitro* and *in-vivo*, can be done to understand these plants' safety profiles better, focusing on long-term use and effects in vulnerable populations.

## Conclusion

This mini-review highlights the potential benefits of *S. aromaticum* and *E. globulus* in improving respiratory infections (RIs). Key findings indicate that *S. aromaticum* contains bioactive compounds such as eugenol, caryophyllene, and eugenol acetate, which have antimicrobial, anti-inflammatory, and antioxidant properties. Similarly, *E. globulus* is rich in eucalyptol,  $\alpha$ -pinene, and limonene, known for their antibacterial, antiviral, anti-inflammatory, and antioxidant effects. These compounds suggest a multifaceted approach to alleviating RIs, supported by both *in-vitro* and *in-vivo* pharmacological studies. The significance of these insights lies in their potential to enhance current RI treatments. Integrating *S. aromaticum* and *E. globulus* into conventional treatment

presents an opportunity to develop more effective management strategies that address the limitations of existing treatments. This integration could be particularly valuable in light of rising antibiotic resistance, providing alternative options that may improve patient outcomes. However, the limitations and gaps should be addressed, especially regarding toxicity issues and unstandardised clinical trials. These plants can potentially become alternative treatments for RIs, and it is essential to understand their safety and effectiveness thoroughly. The findings of this review underscore the need for healthcare practitioners to consider integrating these plants into treatment protocols for RIs. By fostering collaboration between traditional herbal medicine and modern medical practices, the therapeutic options can be enhanced, promoting better health outcomes for patients suffering from respiratory infections.

## Conflict of interest:

The authors have no conflict of interest to declare.

## Authors' contribution:

P. M. R.: Writing - Review & Editing, Project administration; M. N.M.B.: Review & Project planning; N.H.C.B.: Review & Project planning; M. A. A. M.: Software, Formal analysis, Methodology, Writing - Original Draft; M. F. Z.: Formal analysis, Writing - Review & Editing; W. I. W. I.: Writing - Review & Editing;

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Table 1. Bioactive compound and applications of *Syzygium aromaticum* and *Eucalyptus globulis*.

Plant	Compound	Proportion (%)	Applications	Sources
<i>Syzygium aromaticum</i>	Eugenol	74.28	Antimicrobial, insecticidal, anti-inflammatory, wound healing, antioxidant, anticancer (breast, prostate, colon, gastric, and skin cancer)	[25]
	Caryophyllene ( $\alpha$ and $\beta$ )	24.80	Anti-carcinogenic, anti-inflammatory, anxiolytic, antioxidant, anaesthetics effects, potential use as a chemosensitizer	[26]
	Eugenol acetate	2.70	Antimicrobial, anti-inflammatory, antibacterial, use in perfumes because of volatile nature	[25]
	$\alpha$ -copaene	0.17	Antimicrobial, antiproliferative, antigenotoxic, antioxidant, cytotoxic activity	[41,42]
	Methyl salicylate	0.20	Flavouring agent, antibacterial, anti-irritant, antiproliferative	[14, 15]

<i>Eucalyptus globulis</i>	Eucalyptol	60-80	Antibacterial, antifungal, antiviral, anti-inflammatory, cough suppressant, antioxidant and decongestions	[22]
	$\alpha$ -pinene	7-11	Anti-inflammatory, antimicrobial, bronchodilator, cough suppressant	[23]
	Limonene	7	Anti-inflammatory, antioxidant	[25]
	p-cymene	7	Antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal,	[23]
	Globulol	6	Antimicrobial, antioxidant	[23]
	$\beta$ -pinene	3	Analgesic (pain-relieving), anti-inflammatory, antibacterial, antioxidant	[25]
	$\beta$ -myrcene	1-7	Analgesic, anti-inflammatory, antibacterial, antioxidant	[22]
	$\gamma$ -terpinene	1-3	Antioxidant	[24]

Table 2. Studies related to using *S. aromaticum* and *E. globulus* for treating respiratory infections.

Compound	Extract type	Treatment	Results	Ref
<i>S. aromaticum</i>	Clove aqueous extract	Clove extract on the respiratory syncytial virus (RSV) infected HEp-2 cells.	Clove extract exhibited potent anti-RSV activity, appearing virucidal as it directly targeted RSV particles during the early stages of viral infections.	[43]
	Clove mouthwash	Clove mouthwash for the intervention group and chlorhexidine for the control group (twice daily for 5 days) to reduce ventilator-associated pneumonia (VAP) for ICU patients.	The intervention group (clove mouthwash) experienced a reduction in VAP infection compared to the control group. The risk of VAP was 2.06 times higher in the control group than in the clove mouthwash group.	[44]
	Clove essential oil	Clove essential oil on respiratory tract pathogens such as <i>Streptococcus pneumoniae</i> , <i>S. mutans</i> , <i>S. pyogenes</i> , <i>Haemophilus influenzae</i> , <i>H. parainfluenzae</i> , and <i>Moraxella catarrhalis</i> .	Clove oil exhibited antibacterial effects against all tested bacteria, with the strongest inhibition observed on <i>S. pyogenes</i> .	[45]
		Clove essential oil treatment on respiratory infection-related bacteria: <i>Streptococcus pyogenes</i> , <i>S. agalactiae</i> , <i>S. pneumoniae</i> , <i>Klebsiella pneumoniae</i> , <i>Haemophilus</i>	All respiratory infection-related bacterial growths were inhibited when treated with clove essential oil. <i>S. pneumoniae</i> and <i>S. maltophilia</i> showed the highest inhibition.	[46]

		<i>influenzae</i> , <i>Staphylococcus aureus</i> and <i>Stenotrophomonas maltophilia</i>		
		Clove oil on the outer membrane protein of <i>Pseudomonas</i> spp	The outer membrane protein with molecular weights of 42.7 kDa and 79.4 kDa disappeared after treatment which contributes to the antibacterial properties of the clove oil.	[47]
<i>Eucalyptus globulus</i>	<i>Eucalyptus</i> essential oil	Albino Wistar rats acted as the anti-inflammatory, analgesic and antipyretic model (orally treated with the essential oil).	<i>Eucalyptus</i> essential oil showed anti-inflammatory, analgesic and antipyretic effects on the rat models.	[22]
	1,8-Cineole enteric-coated capsules (Soledum®)	Every patient received 90 mg Ingavirin® (antiviral) capsule once daily. In addition, the treatment group also 200 mg received Soledum® capsules (3 capsules a day) for 4–9 days.	Patients treated with Ingavirin® and Soledum® exhibited a significant reduction in cough frequency and other symptoms of acute bronchitis compared to antiviral treatment alone.	[48]
	Isolated eucalyptol	Peripheral blood monocytes isolated from 12 healthy volunteers were used as a model to study inflammatory responses in asthma and chronic obstructive pulmonary diseases.	Eucalyptol reduces the release of inflammatory mediators (IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ ) that cause an inflammatory reaction, suggesting that eucalyptol helps to suppress airway inflammation.	[49]
	Eucalyptol (Soledum®) capsule	Thirty-two patients were randomly assigned to receive	Soledum® treatment reduced steroid dependence in asthma	[50]

	either 200 mg of Soledum® or a placebo for 12 weeks, in addition to the typical steroid treatment for asthma patients.	patients. The capsule also acted as an anti-inflammatory and mucolytic agent in the tested patients.
Eucalyptol gelatin capsule	152 acute rhinosinusitis patients were split into two groups: eucalyptol treatment and placebo.	Patients who received eucalyptol gelatin capsules showed an improvement of over 80% after seven days, compared to the placebo group which showed less than 50% improvement.

[51]

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ORIGINAL ARTICLE

**Zinc and Iron Concentrations in Blood and Organs of Di-(2-ethylhexyl)phthalate (DEHP)-treated Rats.**

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**Abstract**

Oral administration of di(2-ethylhexyl)phthalate (DEHP) to rats is known to induce testicular atrophy and hepatomegaly. This is thought to be the result of increased oxidative stress due to induction of peroxisomal enzymes by mono(2-ethylhexyl)phthalate (MEHP), a metabolite of DEHP. Therefore, changes in the metal content of organs are predicted. Therefore, an experiment was conducted in which rats were fed a diet containing DEHP for two weeks. Metal concentrations in blood and organs were measured, with a focus on zinc and iron. Results are as follows: DEHP treated rats had lower body weight and zinc concentrations in blood, liver, kidney, and testicular tissue, and higher iron concentrations in the liver compared to controls. These changes also correlated with plasma MEHP concentrations, suggesting MEHP-mediated oxidative stress-induced tissue damage and thyroid hormone disruption.

**Keywords:** *di-(2-ethylhexyl)phthalate (DEHP), iron, oxidative stress, zinc.*

## Introduction

Di(2-ethylhexyl)phthalate (DEHP) is a common plasticiser for polyvinyl chloride (PVC), which is used in an extremely wide range of applications, including construction materials, water pipes, household goods, toys, medical devices, blood transfusion tubes, and bags. These PVC products often contain high concentrations of DEHP. Consequently, DEHP is one of the endocrine-disrupting chemicals that people of all ages, from young children to the elderly, are regularly exposed to, raising concerns about contamination and health risks.

It has been reported that feeding rats a diet containing high concentrations of DEHP causes testicular atrophy and hepatomegaly. Testicular atrophy is attributed to the metabolite of DEHP, mono(2-ethylhexyl) phthalate (MEHP), which damages Sertoli cells and induces apoptosis in germ cells [1-7]. Hepatomegaly, on the other hand is caused by MEHP and 2-ethyl hexanol, which cooperate with peroxisome proliferator-activated receptors (PPARs) to activate their peroxisome proliferative effects [8-11], resulting in induction of  $\beta$ -oxidase and cytochrome P450 enzymes [12- 15]. These findings suggest that the toxic effects of DEHP may be due to increased oxidative stress caused by elevated levels of oxidative enzymes, such as heme enzymes, in organs. This oxidative stress could lead to tissue damage via reactive oxygen species (ROS) and alterations in metal levels within tissues due to enzyme induction. Therefore, metal levels in blood and organs were investigated following DEHP administration, focusing on zinc (Zn) and iron (Fe).

## Materials and methods

### Animal experiment

Male Sprague-Dawley rats were purchased from Charles River (Kanagawa, Japan) and fed a CE-2 diet (Clea, Tokyo, Japan) containing 2 w/w% DEHP for the oral administration experiment. The feed was prepared by Oriental Yeast Industry (Chiba, Japan). For the analysis of MEHP by high-performance liquid chromatography

(HPLC), MEHP (purity: 90% or higher) from Tokyo Kasei Kogyo and acetonitrile (HPLC grade) from Wako Pure Chemical Industries were utilised, along with other special grade commercial reagents.

The animal experiment was conducted in accordance with protocols approved by the Kagawa University Animal Committee (No. 123). The rats were housed under the following conditions: a room temperature of 22-24°C, relative humidity of 55-60%, and a light/dark cycle of 12 hours in the animal experiment facility. Five-week-old rats weighing  $168.3 \pm 9.0$  g were divided into two groups, a control group and a treatment group, each consisting of six rats. The treatment group was fed a diet containing 2% (w/w) DEHP for two weeks. At the end of the experiment, rats were sacrificed using ether anesthesia. The testes, liver, and kidneys were removed and weighed. Cardiac blood samples were collected in heparinized tubes and plasma was separated from whole blood by centrifugation at 1500 g. Plasma and organs were frozen at -40 °C until MEHP measurement.

### Blood hemoglobin (Hb) Analysis

Haemoglobin (Hb) concentration was determined using the colorimetric cyanomethemoglobin method with a kit from Wako Pure Chemical Industries, Ltd.

### Metal analysis in blood and organs

Approximately, 200  $\mu$ l of whole blood and 200 mg of organs were taken and placed in a Teflon jar, and 0.5 to 1 ml of mixed acid (nitric acid 50:60% perchloric acid 50: sulfuric acid 1) was added. The mixture was wet ashed at 100-140°C on a hot plate, then heated to 200°C and concentrated. The mixture was evaporated to dryness until white smoke was generated, cooled, diluted with 0.1N hydrochloric acid, and metal analysis was performed by flame atomic absorption spectrometry (1-drop method). The analytical instrument was a Seiko-SAS 7500 model equipped with deuterium background correction (Seiko, Tokyo, Japan).

Zn and Fe content in the animal diets were also analyzed in the same manner. The diets contained 70-80 ppm Zn and 270-310 ppm Fe, with no significant differences in Zn and Fe concentrations between the diets.

### **MEHP analysis in blood and testis**

MEHP in blood and testes was extracted and analyzed by HPLC according to the analysis method previously reported [16].

### **Statistical analysis**

The results were expressed as means  $\pm$  standard deviations (SD). Statistical analyses were performed using a T-test to detect differences between the groups. Differences were regarded as significant at  $P < 0.05$ .

## **Results**

### **Body weights and organ weights**

Table 1 presents the body weights and organ weights for each treatment group. Despite the DEHP group consuming more food than the control group (Figure 1), their weight gain was less than the control group. Figure 2 illustrates a significant negative correlation between plasma MEHP concentrations and final body weight. The DEHP group also exhibited a clear increase in liver weight and a decrease in testicular weight. There was no significant difference in kidney weight. The estimated dose of DEHP (in grams per kilogram per day) based on food intake and average body weight during the treatment period was 1.5 grams per kilogram per day for the DEHP group.

### **Blood metal concentrations**

The blood concentrations of zinc, iron, calcium, and hemoglobin in the DEHP group were found to be slightly lower than in the control group (Table 2). These concentrations demonstrated a statistically insignificant but negative correlation with plasma MEHP concentrations (Figure 3).

### **Organ metal concentrations**

Organ tissue concentrations and organ content of Zn and Fe in liver, kidney and testis are shown in Table 3, Table 4, and Table 5. In the liver, a decrease in Zn concentration and an increase in Fe concentration were observed in the DEHP group. On the other hand, in terms of metal content per organ, there was a clear increase in Fe, whereas Zn did not differ from the control group. In the kidneys, Zn concentrations in the DEHP group were significantly lower than in the control group and the amount of Zn per organ was also lower.

In the testes, Zn concentrations were reduced and Fe concentrations increased in the DEHP-treated group. In terms of metal content per organ, Zn showed a clear decrease, whereas Fe did not differ from the control group.

As shown in Figure 4, the concentration of Fe in liver tissue showed a statistically significant negative correlation with the concentration of MEHP in plasma. Zn concentrations in liver tissue showed a negative, although not statistically significant, correlation with MEHP concentrations in plasma.

As shown in Figure 5, Zn concentrations in kidney tissue showed a statistically significant negative correlation with plasma MEHP concentrations, whereas Fe concentrations in kidney tissue showed no correlation with plasma MEHP concentrations.

Testicular tissue Zn concentrations showed a statistically significant negative correlation with plasma MEHP concentrations, whereas testicular tissue Fe concentrations showed no correlation with plasma MEHP concentrations (Figure 6). However, the concentration of Fe in testicular tissue showed a statistically significant positive correlation with the concentration of MEHP in testicular tissue (Figure 7).

## **Discussion**

MEHP, a metabolite of DEHP, is a potent oxidative stressor, damaging thyroid tissue and lowering thyroid hormones that play an important

role in the process of skeletal muscle formation [19-23]. The suppression of body weight gain and reductions in blood metal and Hb concentrations in the DEHP-treated group observed in this experiment all correlate with plasma MEHP concentrations, implying a disturbance of thyroid function by MEHP.

Furthermore, Zn concentrations in the liver, kidneys and testes of the DEHP-treated group were lower than in the control group and negatively correlated with plasma MEHP concentrations. As nearly 90% of thyroid hormones in the circulating blood are present as the prohormone thyroxine (T4), zinc essential deiodinases in various organs convert this to the biologically active triiodothyronine (T3) [24, 25]. Therefore, there is concern that reduced zinc levels in organs may reduce their ability to activate thyroid hormones, leading to impaired organ function.

Previous studies have reported that testicular atrophy occurs after oral administration of DEHP and that Zn concentrations in testicular tissue are reduced [26-28]. Testicular atrophy also occurs with reduced Zn concentrations in the testis due to Zn deficiency [29, 30]. However, in the case of DEHP-induced testicular atrophy, apoptosis of testicular tissue appears before the Zn concentration is reduced, and Zn administration cannot prevent testicular atrophy [26, 27]. The decrease in Zn concentration is therefore considered to be a secondary phenomenon. In the present experiment, Zn in the testes of the DEHP-treated group was clearly reduced, but the decrease in blood Zn concentration was minor, suggesting that at least DEHP does not affect Zn absorption. Testicular atrophy, on the other hand, occurs when MEHP, a metabolite of DEHP, damages Sertoli cells, which activate the Fas/Fas ligand system and induce germ cell apoptosis [2-5], although the involvement of reactive oxygen species in the Sertoli cell damage process has been suggested [3-7]. In testes with progressive atrophy due to apoptosis induction, a histological picture of loss of sperm, germ cells and other cells is observed (Reference figure). In addition, high

concentrations of Zn have been found to be localised in normal seminiferous tubular tissue [30]. Taken together, the loss of sperm, germ cells, etc. appears to be the major cause of the reduced Zn concentration in atrophied testicular tissue.

There was an increase in the concentration of Fe in the liver and testicular tissue in DEHP-treated group. On the other hand, when converted to Fe content per organ, there was a clear increase in Fe content in the liver, whereas there was no increase in testicular Fe content. Hepatic hypertrophy is based on the coordination of MEHP and 2-ethylhexanol to peroxisome proliferator-activated receptors (PPARs) and activation of their peroxisome proliferative effects [8-15]. This results in induction of  $\beta$ -oxidase and CYP4A [12]. Therefore, it is possible that the large amount of heme enzymes induced in the liver by DEHP administration may partly contribute to the increase in hepatic Fe content, but it is also possible that the heme oxygenase induced by oxidative stress may also promote iron deposition [16-18].

Tissue iron concentrations showed a significant positive correlation with testicular MEHP, even though testicular iron levels in the DEHP-treated group did not differ from those in the control group. This may indicate that iron is unevenly distributed in non-germline supporting tissues and that the relative iron concentration in testicular tissue increases as germline apoptosis progresses.

## Conclusion

This study showed that DEHP-treated rats had lower body weight and zinc concentrations in blood, liver, kidney and testicular tissue, and higher iron concentrations in the liver compared to controls. These changes were also correlated with plasma MEHP concentrations, suggesting MEHP-mediated oxidative stress-induced tissue damage and thyroid hormone disruption. due to MEHP-mediated oxidative stress.

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### Authors contribution

Both authors supervised the laboratory research, analysed results, writing and editing of the manuscript.

### Disclosure of conflict of interest

Authors declare that there is no conflict of interests.

Table 1. Body and organ weights

Group	n	Body (g)	Liver (g)	Kidneys (g)	Testes (g)
Control	6	286 ± 9	14.1 ± 0.8	2.49 ± 0.18	2.49 ± 0.19
DEHP	6	248 ± 26 *	22.1 ± 4.6 ***	2.28 ± 0.38	1.43 ± 0.42 **

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, as compared to control.

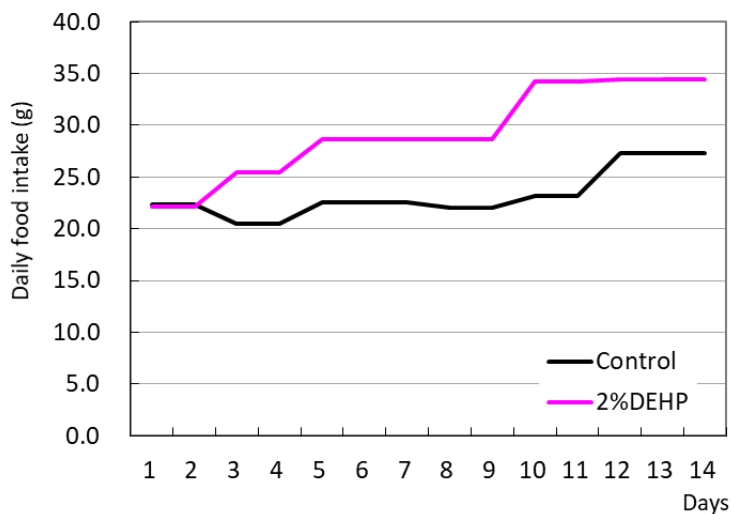


Figure 1. Daily food intake of experimental rats.

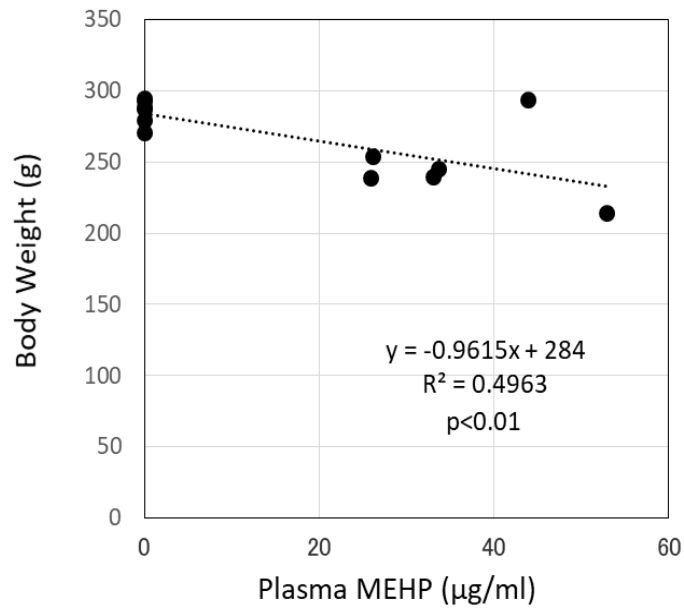


Figure 2. Relation between plasma MEHP concentrations and final body weight

Table 2. Blood zinc, iron, calcium and hemoglobin concentrations

Group	n	Zn (ppm)	Fe (ppm)	Ca (ppm)	Hb (g/dl)
Control	6	4.76 ± 0.60	456 ± 57	44.6 ± 10.2	13.5 ± 0.9
DEHP	6	4.33 ± 0.43	421 ± 39	40.3 ± 10.3	12.7 ± 0.8



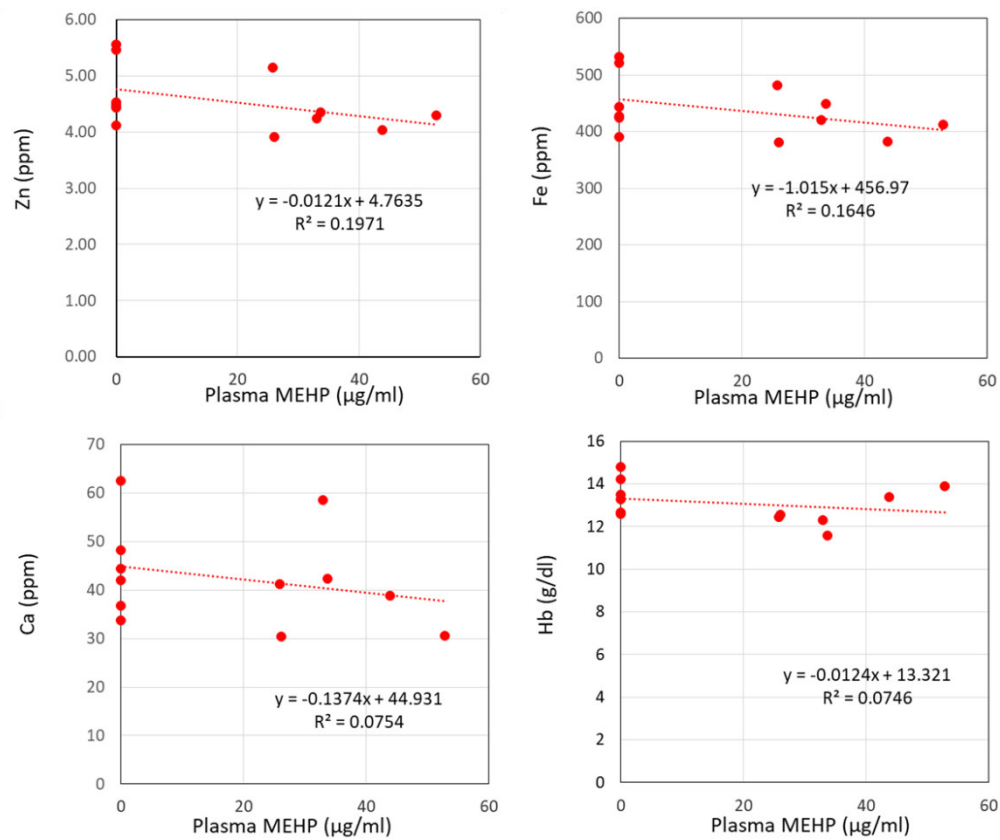


Figure 3. Relation between plasma MEHP concentrations and blood metal and hemoglobin concentrations

Table 3. Tissue concentrations and organ content of Zn and Fe in liver

Group	n	Zn		Fe	
		ppm	µg/organ	ppm	µg/organ
Control	6	22.7 ± 2.7	319 ± 34	54.1 ± 9.5	757 ± 120
DEHP	6	16.9 ± 1.1***	377 ± 105	71.9 ± 12.1	1561 ± 262**

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, as compared to control.

Table 4. Tissue concentrations and organ content of Zn and Fe in kidney

Group	n	Zn		Fe	
		ppm	µg/organ	ppm	µg/organ
Control	6	17.2 ± 5.5	43.4 ± 16.5	57.6 ± 19.4	144 ± 53
DEHP	6	12.2 ± 2.9*	28.6 ± 11.1	58.3 ± 13.3	134 ± 39

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, as compared to control.

Table 5. Tissue concentrations and organ content of Zn and Fe in testis

Group	n	Zn		Fe	
		ppm	µg/organ	ppm	µg/organ
Control	4	20.0 ± 1.3	49.9 ± 7.0	9.9 ± 1.2	24.6 ± 4.2
DEHP	4	14.3 ± 4.7	21.6 ± 11.2*	17.6 ± 6.0	24.1 ± 7.2

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, as compared to control.

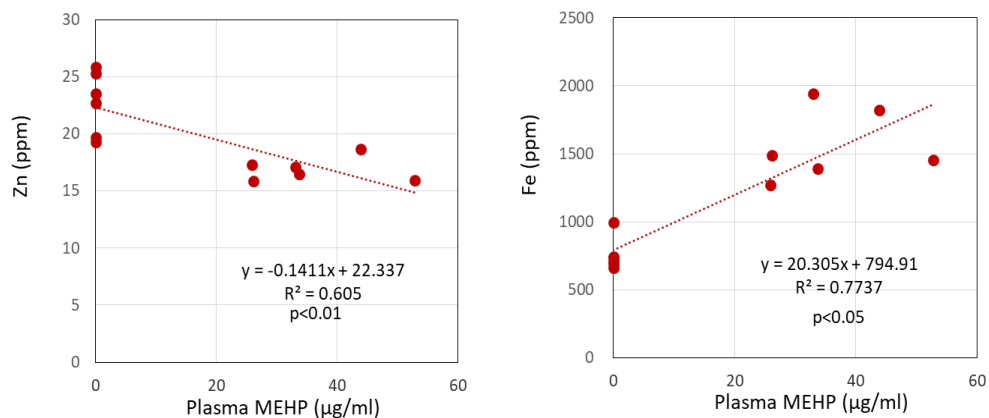


Figure 4. Relation between plasma MEHP concentrations and Zn, Fe concentrations in liver tissue

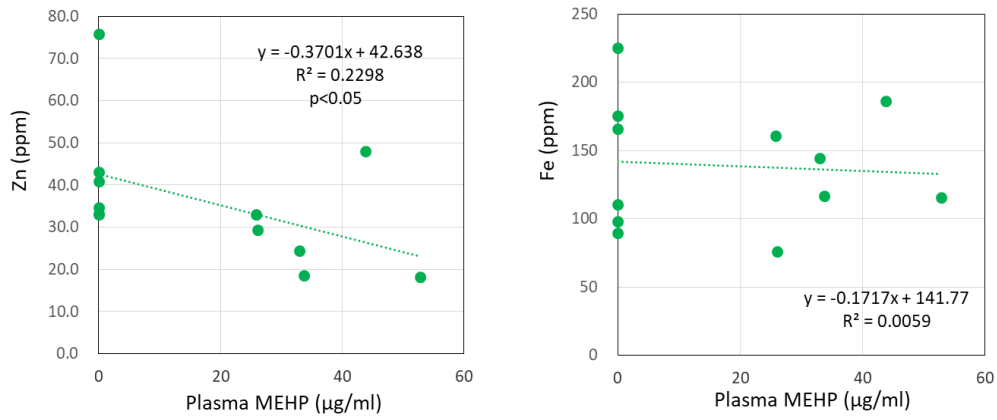


Figure 5. Relation between plasma MEHP concentrations and Zn, Fe concentrations in kidney tissue

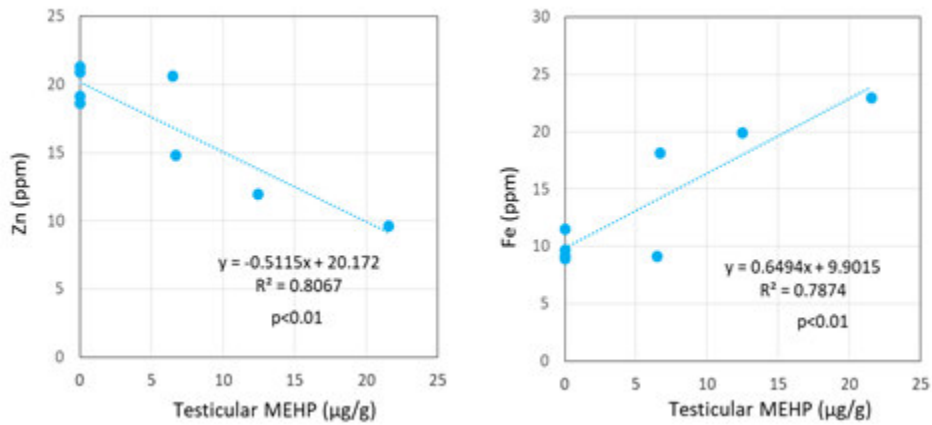


Figure 6. Relation between plasma MEHP concentrations and Zn, Fe concentrations in testicular tissue

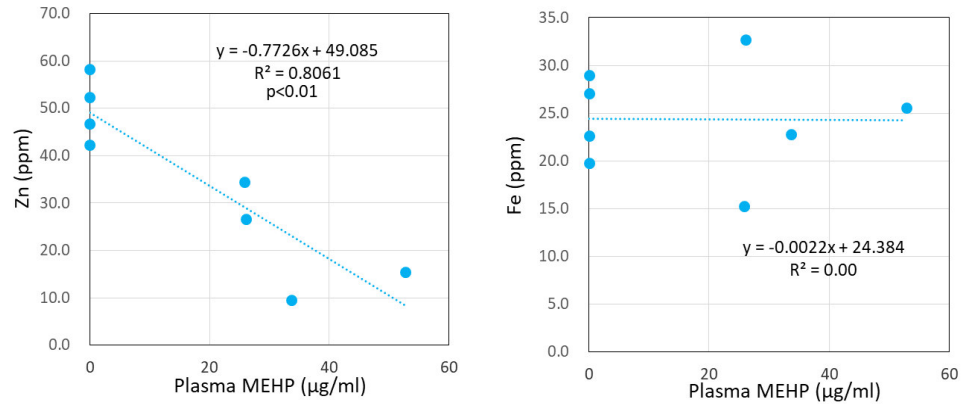
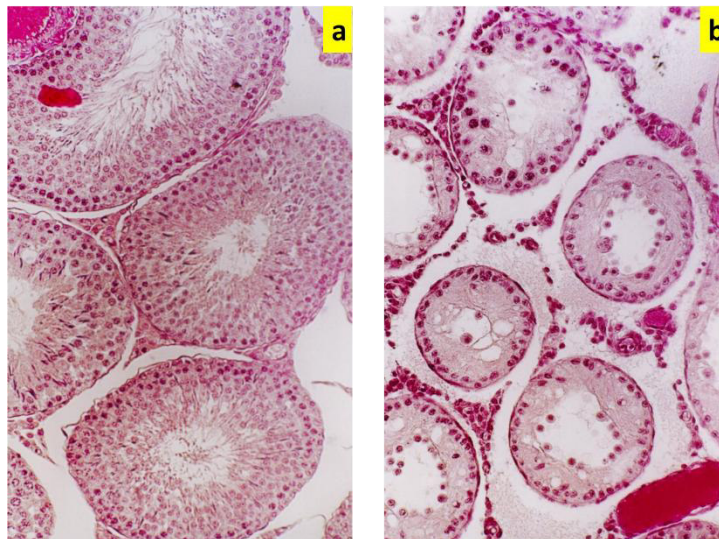


Figure 7. Relation between testicular MEHP concentrations and Zn, Fe concentrations in testicular tissue



Reference figure. Seminiferous tubules of control and DEHP-treated rats (stained with hematoxylin–eosin). a: control, b: DEHP.

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ORIGINAL ARTICLE

**Effectiveness of Yoga Therapy and Pranayama in Reducing Examination-associated Anxiety among (Tenth) 10<sup>th</sup> Standard Students in the Selected High Schools at Bangalore, India.**

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**Abstract**

Anxiety is a human emotion that is a part of life and can often serve as a beneficial form of adrenaline, for instance, before giving a speech, taking an exam, or performing on stage. Anxiety levels vary from student to student, and only a small fraction of exam-anxious students ever receive adequate anxiety reduction training. However, it can drastically impact and affect functions or performance. There are methods to overcome nervousness and anxiety when they interfere with performance. An experimental research design was used in this study on 60, students of 10th-standard from the selected high schools in Bangalore, India This is an experimental research design study conducted on 60 10th-standard students selected from high schools in Bangalore, India. A modified anxiety rating scale was used to assess the level of examination anxiety among 10<sup>th</sup>-standard students. Pre-test- and post-test data were collected after 4 weeks of intervention on both the experimental and control group. Yoga and pranayama were given to the experimental group along with counselling, whereas only counselling was given to the control group. The results of this study showed that in the experimental group, the overall mean pre-test and post-test scores were 100.02 and 54.01, respectively, with a paired 't' value of 11.60, which was above the table value of 2.7 at  $p \leq 0.01$  level of significance. Whereas in the control group, the overall mean pre-test and post-test mean score was 104.77 and 101.43 respectively with paired 't' value of 0.82, which is not significant at  $p \leq 0.01$  level of significance. Excessive exam anxiety can result in stress and may negatively affect performance. Yoga therapy may help individuals work with the physical, as well as the emotional tensions that either reduce anxiety.

**Keywords:** *anxiety, exam, pranayama, yoga.*

## Introduction

Anxiety is a human feeling that is part of life and can often serve as a good form of adrenaline for instance, before making a speech, taking an exam, or performing on stage. However, there are methods on how to overcome nervousness and anxiety when they interfere with performance. Exam anxiety can also be experienced at varying levels. Slight exam stress can help by providing alertness, readiness, and helping you to concentrate. However, excessive exam anxiety can result in stress and negatively affect performance. Exam anxiety, just like other types of anxiety, tends to occur like a wave. It will increase from the time you first recognize it, come to a peak, and then naturally subside [1].

The flawed examination system and the highly competitive environment governed by the market forces, create tremendous competition for every job and profession, placing a great amount of strain on individuals appearing for exams [2].

Strangely, stress levels peak to a crescendo, just before the dreaded 'Board exams' in class 10 and 12. There is a great emphasis on academic excellence, as it is a gateway to a future of bright career prospects.

Often, even parents get caught in this vortex of exam stress, further heightening their child's tension with undue expectations. As a result, children feel double the pressure so as not to let their parents down [3].

Many students experience anxiety while undertaking exams. There are four main areas of stress which can contribute to exam anxiety: lifestyle issues, lack of required information, studying style, and psychological factors [4].

Students face a lot of pressure in school. Some of the excellent sources of stress and anxiety of students are the dreaded examinations and evaluation tests. Usually, the anxiety felt by students serves as a good motivator to study and perform well. However, there are times when some students worry more than they should really worry regarding their exams. Various studies showed that exam anxiety affects almost 50% of students in India. The prevalence rates range from 4.0% to 25.0%, with an average rate of 8.0% [5].

More research indicates that more than 33% of US elementary and secondary students experience some exam anxiety [6].

Keeping in view the above findings in the literature, we believe that droves of students have exam-related anxiety, particularly among SSLC students. These students struggle significantly with academic activities. If this anxiety is left untreated, performance anxieties continue into adulthood, hindering career choices and lowering the quality of life. Anxiety levels vary from student to student, and only a small fraction of exam-anxious students ever receive adequate anxiety reduction training. Therefore, it is important to measure the anxiety levels in different students and provide proper interventions to resolve it. The goal is to reduce symptoms of examination anxiety, which can lead to problems such as school dropouts, running away, depression, or even suicide, and to help the students face the board exams without distress. For this reason, yoga and pranayama are used as methods to reduce examination anxiety among the 10<sup>th</sup>-standard students.

This study aimed to assess the level of examination anxiety of 10<sup>th</sup>-standard students before implementing yoga therapy and pranayama in experimental and control group and also to evaluate the effectiveness of yoga therapy and pranayama in reducing examination anxiety among them.

## Methods

### Research design

An experimental research design was used for conducting this study.

### Sample and sample size

The sample size of the study was 60, students of 10<sup>th</sup>-standard students from selected high schools in Bangalore.

### Sampling technique

Simple random sampling technique was used to select the samples of this study. Sixty students of 10<sup>th</sup>-standard from the selected from high schools in Bangalore, India Bangalore were

selected. On the first day, the 10th standard students who met the inclusive criteria were selected and their names were recorded. Samples were selected on consecutive days until the sample size reached 60.

#### **Inclusion criteria**

The study included 10th standard students, who:

- Were studying in selected high schools Bangalore.
- Able to do yoga and pranayama
- Willing to participate in the study.
- Able to read and write in English or Kannada

#### **Exclusion criteria**

The study excluded 10th standard students, who:

- Participated in the pilot study
- Were not available during data collection.
- Were specially challenged.

#### **Data collection procedure**

Prior to the study ethical approval was obtained from the institutions' ethical committees. The data was collected from 10th-standard students in Delhi Public School, Bangalore. Written permission was sought and obtained from the authorities concerned. The period of data collection was 4 weeks. Informed consent was taken from each participant and the purpose of the study was explained to them. Students were made comfortable, and privacy was taken into account while conducting this study. Instructions to answer the questionnaire were given. A pre-test was conducted through a modified anxiety rating scale to assess the level of examination anxiety among the participants. Yoga therapy and pranayama were explained and demonstrated to the experimental group along with counselling, and only counselling was given to the control group. Yoga and pranayama were carried out three days a week for four weeks under the supervision of a yoga trainer. After 14 days of initiating the intervention, a posttest was conducted for participants, in both the experimental and the control group to assess their reduction in exam anxiety.

#### **Data analysis**

The data obtained was analyzed using descriptive and inferential statistics. Mean and standard deviation were used to assess the pre-test and post-test level of examination anxiety among 10th standard students in experimental and control group. Paired 't' test was used to evaluate the effectiveness of yoga therapy and pranayama in reducing examination anxiety among 10<sup>th</sup> standard students at  $P \leq 0.01$  level of significance.

#### **Results**

##### **Socio-demographic variables**

Table 1 shows demographic variables such as age, gender, religion, type of family, area of residence, family monthly income, personal history of anxiety.

##### **Anxiety level**

Table 2 depicts the pretest level of examination anxiety among 10th standard students in experimental and control group. Among 30, students in experimental group majority 18(60%) of them had severe level and 12(40%) of them had moderate level of examination anxiety, whereas in the control group 22(73.33%) had severe and the remaining 8 (26.67%) of them had moderate level of examination anxiety.

Table 3 describes the mean pre-test scores of examination anxiety among students in experimental and control group. It was observed that, 10th standard students in experimental group had mean pre-test examination anxiety of 100.02 with SD 13.07 whereas in the control group mean pre-test examination anxiety and SD were 104.77 and 12.09 respectively.

Table 4 illustrates post-test level of examination anxiety among 10th standard students in experimental and control group. Among 30 10th-standard students in experimental group, majority 23 (76.67%) had a normal level, 7 (23.33%) of them had moderate level, whereas in control group 17 (56.67%) of them had severe level, 13 (43.33%) of them had a moderate level of examination anxiety.

Table 5 describes a mean post-test score of examination anxiety among 10<sup>th</sup> standard students in experimental and control group. It was observed that, 10<sup>th</sup> standard students in experimental group had mean post-test examination anxiety score of 54.01 with SD 7.64 whereas in control group mean post-test examination anxiety score and SD were 101.43 and 11.39 respectively.

### **Yoga and Pranayama**

Table 6 depicts the effectiveness of yoga therapy and pranayama in reduction of examination anxiety among 10<sup>th</sup> standard students. It is inferred that, in the experimental group, the overall paired 't' test value was 11.60, which is significant to the table value 2.7 at  $p \leq 0.01$  level. This proves that yoga therapy and pranayama were effective in reducing examination anxiety among 10<sup>th</sup> standard students. In contrast, in the control group, the overall paired 't' test value was 0.82, which is not significant to the table value 2.7 at  $p \leq 0.01$  level, indicating that there is no change in examination anxiety among the control group.

### **Discussion**

#### **To assess the level of examination anxiety of 10<sup>th</sup> standard students before implementing yoga therapy and pranayama**

It was observed that, in pre-test, among 30 students in experimental group majority 18 (60%) had severe and 12 (40%) of them had moderate level of examination anxiety with mean score of 100.02 whereas in control group 22 (73.33%) of them had severe and remaining 8 (26.67%) of them had moderate level of examination anxiety with mean score of 104.77.

The finding of the present study is supported by the findings of the following studies.

A study conducted on a group of 100 students-50 boys and 50 girls from 10<sup>th</sup> and 12<sup>th</sup> grades - aimed to assess the Test Anxiety Levels of Board Exam Students in Tamil Nadu, India, before the board exams. The study discussed exam-related anxiety, finding that among the boys, 8% had

severe, 38% recorded moderate, and 4% had mild anxiety. On the other hand, severe anxiety was not found among girls. The significance of difference between the mean value of male and female students was calculated by independent sample - test and the existing difference was found to be statistically significant. Levene's test showed that the variability among males and females is more or less the same (value = 0.06). [7].

The findings of the present study as well as previous studies showed that students had severe levels of examination anxiety and measures should be undertaken to reduce their anxiety level. In this study a comparison was made between the pre-test and post-test mean scores to evaluate the effectiveness of yoga therapy and pranayama in reducing examination anxiety among 10<sup>th</sup> standard students. It was observed that, in the experimental group the overall mean pre-test and post-test mean scores were 100.02 and 54.01 respectively with paired 't' value of 11.60, which was above the table value 2.7 at  $P \leq 0.01$  level of significance. Hence the research hypothesis H1 is accepted. Whereas in control group, the overall mean pre-test and post-test mean score was 104.77 and 101.43 respectively with paired 't' value of 0.82, which is not significant at  $P \leq 0.01$  level of significance.

In an experimental study conducted at Azad University, Iran to evaluate the influence of pranayama in relieving symptoms of test anxiety in 107 postgraduate Iranian students the researchers found average prevalence of test anxiety in the experimental group pre- and post-yoga intervention was  $18.48 \pm 5.68$  and  $16.00 \pm 4.81$ , respectively. Researchers have used the convenience sampling method and randomly assigned the participants into experimental and control groups. The study concluded that pranayama could lead to a significant reduction in perceived levels of anxiety. The researcher suggests that pranayama can be considered for a nervous student or anyone who is familiar with how anxiety manifests itself in the body and mind [8].

The present study and previous studies clearly showed that yoga therapy and pranayama were effective in reducing examination anxiety among 10<sup>th</sup> standard students.

### **Effectiveness of yoga therapy and pranayama in reducing examination anxiety among 10<sup>th</sup> standard students**

In this study, a comparison was done between the pre-test and post-test mean scores to evaluate the effectiveness of yoga therapy and pranayama in reducing examination anxiety among 10<sup>th</sup> standard students.

The finding of the present study is supported by the findings of the following studies.

An experimental study was conducted in Kendriya Vidyalaya, Agra India to evaluate the influence of pranayama on academic performance of 9<sup>th</sup> and 11<sup>th</sup> standard students. A sample of 50 students (28 boys, 22 girls) was selected using convenience sampling and randomly assigned. The study findings revealed that the average prevalence of performance post-pranayama intervention was 77.82±11.5 and 99±10.1, respectively. When compared with pre and post, girls who participated in pranayama classes showed significant improvement in performance from 77.7±10.9 to 98±11 and boys were from 77±11.9 to 99.6±11. The study concluded that pranayama could lead to a significant reduction in perceived levels of anxiety. The researcher suggests that pranayama can be considered as a complementary therapy for improving their academic performance [9].

In another study conducted in a private yoga clinic, India to find the effect of yoga on depression and anxiety in women. The average prevalence of depression in experimental group pre and post-yoga intervention was 12.82+/-7.9 and 10.79+/-6.04, respectively, a statistically insignificant decrease (p=0.13). However, when compared the experimental and control group post intervention showed a decrease in state anxiety (p=0.03) and trait anxiety (p<0.001). This researcher suggests that yoga can be considered as a complementary therapy or as an alternative

method for medical therapy in the treatment of anxiety disorders [10].

The present study, along with previous studies clearly showed that yoga therapy and pranayama were effective in reducing examination anxiety among 10<sup>th</sup> standard students.

### **Conclusion**

This study demonstrated that interventions involving yoga and pranayama can effectively reduce examination anxiety. These methods are worth considering as alternative therapies for managing anxiety. Future research should explore whether reducing anxiety through these techniques has any impact on students' exam performance.

### **Limitations**

This study is delimited to:

- The assessment of the level of examination anxiety was based only on the correct responses given to the items in the anxiety scale.
- Data were Collected only from 10<sup>th</sup> standard students in selected high schools, Bangalore, India.
- 60, 10<sup>th</sup> standard students.
- 4 weeks of data collection.

### **Recommendations**

- Study may be conducted by comparing the students living in hostel, in a home can have different methods of coping.
- Study may be conducted for the students involved in physical activity / using relaxation methods / listening music etc.
- Study may be conducted by comparing the 10<sup>th</sup> and 12<sup>th</sup> standard students to find the effect of yoga and pranayama.

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### **Conflict of Interest**

None

**Authors contribution**

JAM- Main researcher conducted research, and draft original manuscript; VSM- Co-researcher, reviewed, edited, and finalized manuscript.

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None

Table 1. Frequency and percentage distribution of selected socio-demographic variables of 10<sup>th</sup> standard students.

Characteristics	Category	Respondents			
		Experimental Group		Control Group	
		N	%	N	%
Age	15 years	20	66.67	26	86.67
	16 years	7	23.33	4	13.33
	17 years or above	3	10	0	0
Gender	Male	18	60	7	23.33
	Female	12	40	23	76.67
Religion	Hindu	16	53.33	10	33.33
	Muslim	6	20	8	26.67
	Christian	8	26.67	12	40
	Others	0	0	0	0
Area of residence	Urban	23	76.67	30	100
	Rural	7	23.33	0	0
Family monthly income	Rs. 5000 or below	1	3.33	0	0
	Rs. 5001-15000	9	30	3	10
	Rs. 15001-25000	14	46.67	8	26.67
	Rs. 25001 or above	6	20	19	63.33
Supporting system	Family members	24	80	29	96.67
	Relatives and friends	5	16.67	1	3.33
	Neighbors	1	3.33	0	0
	Nil	0	0	0	0
Personal history of anxiety	Yes	2	6.67	29	3.33
	No	28	93.33	1	96.67

Table 2. Pre-test level of examination anxiety among 10th standard students in experimental and control group.

Level of Anxiety	Score	Respondents			
		Experimental Group		Control Group	
		N	%	N	%
Low level of anxiety	0-50	0	0	0	0
Moderate level of anxiety	51-100	12	40	8	26.67
Severe Level of anxiety	101-150	18	60	22	73.33
Total		30	100	30	100

Table 3. Mean pre-test score of examination anxiety among 10th standard students in experimental and control group

Area wise anxiety assessment	Max Statement	Max Score	Experimental Group			Control Group		
			Range	Mean	SD	Range	Mean	SD
Assessment of stressors	15	45	8-42	29.85	8.95	12-44	31.09	7.38
Physical Symptom	8	24	4-23	16	4.86	6-21	15.57	4.90
Emotional symptom	14	42	9-42	28.5	8.7	8-43	30.60	7.81
Mental symptom	7	21	8-21	14.45	4.06	10-21	14.01	3.93
Behavioral symptom	6	18	3-18	11.21	3.7	5-18	12.69	3.15
Overall	50	150	32-146	100.02	13.07	41-147	104.77	12.09

Table 4. Post-test level of examination anxiety among 10th standard students in experimental and control group.

Level of Anxiety	Score	Respondents			
		Experimental Group		Control Group	
		N	%	N	%
Low level of anxiety	0-50	23	76.67	0	0
Moderate level of anxiety	51-100	7	23.33	13	43.33
High Level of anxiety	101-150	0	0	17	56.67
Total		30	100	30	100

Table 5. Mean post-test score of examination anxiety among 10th standard students in experimental and control group

Area wise anxiety assessment	Max Statement	Max Score	Experimental Group			Control Group		
			Range	Mean	SD	Range	Mean	SD
			Assessment of stressors	15	45	2-32	17.08	6.33
Physical Symptom	8	24	4-13	8.62	3.04	4-22	15.37	4.24
Emotional symptom	14	42	3-23	14.61	5.61	5-41	29.81	6.91
Mental symptom	7	21	2-16	7.31	2.94	9-20	13.64	3.47
Behavioral symptom	6	18	0-13	6.39	1.92	5-19	12.32	3.04
Overall	50	150	11-97	54.01	7.64	35-141	101.43	11.39



Table 6. Effectiveness of yoga therapy and pranayama in reduction of examination anxiety among 10th standard students

Group	Aspect	BP of Respondents		Paired 't' test
		Mean	SD	
Experimental Group	Pre-test	100.02	13.07	<b>11.60**</b>
	Post-test	54.01	7.64	
Control Group	Pre-test	104.77	12.09	<b>0.82</b>
	Post-test	101.43	11.39	

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ORIGINAL ARTICLE

## Exploring Workflow Gaps in Diabetic Foot Care: Insights from Primary Care Clinics in Kuantan.

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### Abstract

The diabetic foot is a common complication of diabetes. According to the latest guideline on the management of diabetic foot (2018) from the Ministry of Health, it was suggested that primary care clinics develop foot protection teams to reduce hospital admissions, length of stay, and the amputation rate. However, there are barriers to establishing these teams. This research aimed to explore the current workflow for managing diabetic foot in primary care clinics in Kuantan. A purposive sample of 12 healthcare professionals was selected for this qualitative research project from four Kuantan primary care clinics with the highest number of recorded patients in the National Diabetes Registry. Semi-structured, focus group interviews were conducted via an online platform. Interviews were recorded and transcribed verbatim. The data were analysed via thematic analysis. The study identified three main themes: workflow, healthcare provider roles, and guideline implementation. Clinics lacked standardized workflows regarding operating hours, dedicated teams, and improper screening practices, which often deviated from established guidelines. The screening tools were outdated and not aligned with current guidelines. Guideline implementations appear lacking among healthcare providers mostly due to ignorance of the availability of the latest Clinical Practice Guideline (CPG). Before a diabetic foot protection team can be successfully built, several obstacles must be addressed. One potential project is the development of a clear workflow algorithm that can be employed in clinic settings.

**Keywords:** *diabetic foot, foot protection team, foot care services, primary care clinics, Malaysia.*

## Introduction

Diabetes mellitus imposes a significant global health burden, with foot complications being particularly severe and costly. In Malaysia, over two million individuals aged 20 to 79 had diabetes in 2011, a figure projected to increase by 50% by 2030 [1]. This rise is expected to coincide with more diabetic foot complications. Data from the 2020 National Diabetes Registry highlights persistent diabetic foot ulcers and amputations, which continue to strain healthcare systems [2]. Diabetic foot issues cause considerable mortality, morbidity, and financial burdens, with three-quarters of amputations in Malaysia linked to diabetes. A tertiary care facility on the East Coast reported an 11% incidence of major limb amputations among diabetic patients in 2013, with diabetics facing a 12.3 times higher risk of amputation than the general population [3]. The economic impact is significant, with acute diabetic foot infections costing RM 32,000 annually per patient [3,4]. Diabetic foot complications also severely affect quality of life, with major contributing factors including trauma, neuropathy, deformity, poor knowledge, inadequate foot care, prolonged diabetes duration, and smoking [5-7].

To address these challenges, the Ministry of Health (MOH) of Malaysia has formulated a Diabetes Foot Care Model to enhance patient care and facilitate seamless referral across healthcare tiers. However, significant barriers hinder effective implementation, necessitating a cohesive multidisciplinary approach involving endocrinologists, primary care physicians, diabetes nurse educators, dietitians, pharmacists, and podiatrists. Despite the presence of trained professionals, healthcare services remain fragmented, necessitating coordinated efforts to enhance patient access.

Data from the MOH underscores the predominance of primary care clinics as the initial point of contact for diabetic patients [8]. While new guidelines propose the establishment of foot protection service teams within primary care settings, clarity regarding team composition, responsibilities, and referral criteria remains

lacking. According to the international guidelines, the Foot Protection Service aims to prevent and treat simple active diabetic foot problems within the community, thereby averting complications that may lead to amputation [9]. According to the current Clinical Practice Guideline (CPG), this service should be led by a Family Medicine Specialist or a physician with specialized training in diabetic foot issues, complemented by a team consisting of podiatrists, diabetic educators, wound care specialists, and rehabilitation experts [10]. Evidence suggests that establishing such a service in primary care settings can significantly enhance outcomes for diabetic foot complications. A systematic review in 2019 demonstrated that the implementation of a dedicated multidisciplinary team effectively reduces the incidence of major amputations due to diabetic foot ulcers [11].

Hence, this study assumes significance in laying the groundwork for a structured framework to optimize primary care foot protection services, thereby enhancing diabetic foot care management and guideline adherence.

## Research Methodology

### Study design, population and sampling method.

This qualitative study used focus group discussions involving twelve healthcare professionals from four clinics with the highest number of diabetic patients, selected from the National Diabetes Registry. The chosen clinics were KK Bandar Kuantan, KK Beserah, KK Jaya Gading, and KK Paya Besar all having diabetic nurses, Medical Officers (MO), Family Medicine Specialists (FMS), and wound care services. Purposive sampling ensured participants met specific expertise criteria [9,10]. Respondents must have had over six months of experience in diabetic foot care and were proficient in English or Malay. Those who were on maternity leave, study leave, or working in other units were excluded. Data collection occurred between September 2021 and June 2022.

### **Data collection**

Amidst the pandemic, virtual focus group discussions with twelve healthcare providers were conducted via Zoom or Google Meet. Participants were split into three groups: diabetic nurses, medical officers (MO) in charge, and Family Medicine Specialist (FMS), with four members each representing their clinics. Identified as interviewees A (diabetic nurse), B (medical officers), and C (FMS), each session lasted 90-120 minutes using semi-structured questions. A semi-structured interview guide was developed to address the objectives of the study. The interview guide was pre-tested to the members of the study population that was not involved in this study to ensure clarity and refinement of the questions before actual data collection. The information gathered from the focus group interview was both comprehensive and sufficiently rich to address the research objectives.

### **Interview**

All the interviews were carried out by a researcher who was a trainee in the Family Medicine postgraduate program that had attended training in qualitative study. The researcher had not worked in the clinics being studied and had no affiliation with the participants. Respondents received an information sheet detailing the study's nature and purpose and gave informed consent before the interview. The interviews were conducted online, with a video inspection of the participants' rooms to ensure that only the intended participants were present during the interview. Prior to the interviews, recording consent was obtained, and participants were briefed on the study's structure. If interviewees made unclear statements, the interviewer intervened to seek clarification. Throughout the interview, respondents were encouraged to share additional insights or discuss topics they felt had been overlooked. At the end of the session, the interviewer expressed gratitude and cross-checked participants' thoughts. Interviews were transcribed verbatim in either Malay or English

immediately after the session, with field notes maintained to ensure context preservation.

### **Data Analysis**

Thematic analysis was used to examine patterns in the data. NVIVO 12 was employed to code interview transcripts and categorize them into common themes. The research team discussed and finalized these codes and themes. Quotations were translated into English to illustrate each theme. The analysis followed Braun and Clarke's six phases: data familiarization, initial coding, exploration, labelling, theme review, and report compilation.<sup>12</sup> Broader patterns were identified as superordinate themes, with specific categories termed sub-themes.

### **Ethical Consideration**

Ethics approval was provided by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia, reference NMRR-21-1252-60118. Written informed consent was obtained before the interviews.

### **Results**

#### **Sociodemographic characteristics of the respondents**

The total number of respondents was twelve. The respondents' ages range from 31 to 55 years old, with a mean age of 43. The respondents consisted of four FMSs, four MOs and four diabetic nurses. All were Malays except for one Chinese.

#### **Current structures in diabetic foot care service**

The primary aim of this study was to examine the existing framework for diabetic foot management in primary care clinics in Kuantan, Malaysia. Three key themes emerged to elucidate this framework: the workflow of diabetic foot care, the roles of healthcare providers, and the implementation of current guidelines in foot care.

## Theme 1: Workflow of diabetic foot care

Three subthemes were identified within this theme: "Operating hours for diabetic patients," "Absence of a dedicated foot care team," and "Diabetic foot screening conducted only for symptomatic patients."

### **Operating hours for diabetic patients**

The study revealed variations in operating hours for diabetic patients across the four primary care clinics. Some clinics designate specific days for diabetic patient consultations, such as Tuesdays: "We have a specific day to see the DM cases which is every Tuesday." (A3)

Others see diabetic patients daily:

"We divided to outpatient and NCD clinic. Hence no specific day to see the DM cases. The doctor was allocated according to this." (A1, A2, A4)

### **The absence of a dedicated foot care team**

Data from this study revealed the absence of a dedicated foot care team in primary care clinics. Respondents across various clinics concurred with this finding:

"There is no specific team in managing the diabetic foot. They usually will be managed by the wound care team if they develop any wound or ulcer." (C4)

### **Diabetic foot screening is conducted only for symptomatic patients**

This subtheme highlights that diabetic foot screening is conducted solely when patients exhibit symptoms. Respondents provided insights on this practice:

"Usually, we will ask if they have any symptoms of diabetic foot. Then, we will examine their feet." (A1)

## Theme 2: Role of healthcare provider in diabetic management.

This theme delves into the responsibilities of healthcare providers in addressing diabetes management within primary care clinics. Three distinct sub-themes have emerged: the involvement of various healthcare providers in

diabetic screening and the specific roles of both medical officers and family medicine specialists.

### **Different healthcare providers do the screening.**

The findings of the study indicate that diabetic foot screening was conducted by various healthcare providers, predominantly by nurses. Respondent A1 asserted,

"Typically, all screening procedures are carried out by us" (A1).

In contrast, Respondent A3 disclosed,

"In our clinic, a similar approach is adopted, albeit with the assistance of occupational therapists who aid in conducting diabetic foot screenings" (A3).

### **Medical officer role:**

Other responses indicate that medical officers typically attend to patients after they have been seen by nurses or if any issues arise during the screening process. For instance, Respondent B4 stated,

"Nurses conduct the screening, after which we assess the patient" (B4).

Respondent B3 added,

"We evaluate the patient, provide treatment, and primarily focus on educating them about medication adherence, sugar control, and diet management" (B3).

Similarly, Respondent A1 expressed,

"We handle the screening procedures, and if any complications arise, we refer the patient to a doctor. The doctor then assesses, treats, and refers if necessary" (A1).

### **Family Medicine Specialist role:**

This subtheme elucidated the role of Family Medicine Specialists (FMS) in the management of diabetic patients. As articulated by Respondent C1,

"Medical officers initially assess the patient, and if necessary, they refer the patient to me for additional management. My responsibilities include overseeing clinic operations and conducting audits to ensure adherence to diabetes care guidelines."

This perspective was corroborated by other respondents C2 and C3.

### **Theme 3: Implementation of the latest guidelines in diabetic foot care.**

This theme focuses on the utilization of the latest guidelines for foot care in primary care settings. It comprises three subthemes: reliance on the diabetic book as a reference, lack of awareness regarding the latest foot care guidelines, and the availability of the current Clinical Practice Guidelines (CPG) as a reference in clinics.

#### ***Reliance of the diabetic book as a reference.***

Regarding the use of the diabetic book as a reference, Respondent A4 stated,

*"We utilize the diabetic book for screening purposes as it is readily available in the clinic and easily accessible. Subsequently, we document the findings in the book."*

This viewpoint garnered agreement from other participants. Similarly, Respondent B1 noted,

*"In my clinic, nurses conduct the screening, and I've observed they utilize a diabetic book."*

This perspective was also supported by other respondents.

However, Respondent C1 expressed disagreement, emphasizing,

*"Screening should adhere to the checklist outlined in our CPG, followed by risk stratification. This approach enables appropriate management of diabetic foot issues based on the patient's risk level."*

This stance was shared by other respondents (C2, C3, and C4).

#### ***Lack of awareness regarding the latest foot care guidelines.***

Several respondents were unaware of the existence of the latest Malaysian Clinical Practice Guidelines (CPG) for diabetic foot management. Respondent A2 expressed surprise, stating,

*"I wasn't aware of a new CPG for diabetic foot. I've never come across it."*

This sentiment was echoed by Respondents A1 and A4, who stated,

*"Oh, is there a new one? We only have the old version in our clinic. Therefore, we refer to the*

*old CPG if there's uncertainty in screening and management."*

#### ***Availability of the current CPG as a reference***

While some respondents were unaware of the latest foot care guideline, others are cognizant of it but lack access to it for managing diabetic foot issues.

Respondent B3 stated that,

*"I'm aware that the guideline includes a checklist for diabetic foot screening, but it's not accessible in our clinic. Therefore, I rely on what's available, like the diabetic book."*

### **Discussion**

Understanding the current framework for managing diabetic foot is essential for improving services and outcomes. This study identified three key themes: workflow, healthcare provider roles, and guideline implementation. The analysis revealed a lack of standardization in operating hours for diabetic patients across the four primary care clinics studied. Some clinics designated specific days for diabetic patients, while others provided services every weekday. This variability aligns with a survey by Mustapha et al. (2020), which highlighted differences in the number of days clinics allocated for diabetes appointments, ranging from 2 to 5 days a week [12]. This variation may be attributed to staffing levels and patient burden.

Additionally, the absence of dedicated diabetic foot care teams was observed. This finding aligns with a study by Hussein et al. (2015), which noted disparities in diabetes teams among clinics. Some had dedicated teams led by trained personnel, while others did not. Variations were due to community diversity and logistical differences between urban and rural clinics, with urban clinics better equipped [8]. Generally, Malaysian primary healthcare settings lacked dedicated diabetes teams, possibly due to high disease burden and staff shortages, leading to multitasking, inefficiency, and suboptimal care [13].

The screening of diabetic foot conditions plays a crucial role in preventing problems among diabetic patients, and it is recommended to conduct such screenings at least annually for diabetic individuals [14,15]. Surprisingly, this study found that diabetic foot screenings are typically only performed when patients exhibit symptoms, aligning with the findings of a study by Ranuve et al (2022). In that study, healthcare workers (HCWs) acknowledged that they do not routinely provide foot care advice to diabetic patients without diabetic foot ulcers (DFU) during clinic visits. Foot care advice is typically given only to those who have already developed DFU. Some respondents in the study mentioned that they may defer foot assessments if patients attribute symptoms like numbness to factors such as cold weather [16,17]. This approach poses a serious concern, as many diabetic foot complications stem from peripheral neuropathy, leading to a loss of protective sensation. Therefore, regular foot screenings for all diabetic patients, regardless of symptoms, are essential. Husna et al.'s study supports this approach, revealing that patients with a foot at risk often lack knowledge and awareness of diabetic foot care [6,7]. Healthcare providers must prioritize regular screenings and educate diabetic patients on foot care to enable early prevention and detection of potential issues. It is crucial to address challenges such as a shortage of resources, including insufficient staff, multitasking demands, and a high volume of diabetic patients. This situation warrants attention from superiors and policymakers to ensure effective and optimal care for diabetic patients.

A noteworthy finding in this study is the diverse roles of healthcare providers in diabetic foot care. Foot screening is often conducted by various professionals, predominantly nurses. Some clinics have occupational therapists and rehabilitation staff for screenings, while others lack such specialized personnel. The presence of diabetic educators varies, with the Ministry of Health (MOH) survey indicating that only 6 out of 10 clinics have these professionals. These

educators often have additional duties like managing screening counters and reviewing blood investigation results. The study highlights nurses' responsibilities in conducting foot screenings and referring patients to medical officers when problems are identified. This aligns with a survey by Feisul Idzwan Mustapha et al., which found that nurses ensure the completeness of screening for diabetes-related complications and trace blood investigation results [12]. Medical officers play a crucial role in examining referred patients, providing treatment, educating on diabetic foot care, and making necessary referrals. Feisul's survey also noted that medical officers in some clinics are responsible for the complete documentation of medical records [12]. The implementation of the latest guideline is another aspect supporting the current practices of healthcare workers in Kuantan, Malaysia. Findings from this study suggest that guidelines for treating diabetic foot have not been fully integrated into clinical practice among the respondents involved. Similar results were seen in a study across four Nordic countries, where only 39% of respondents reported using the guidelines, despite their introduction over 15 years ago [18]. Factors influencing the adoption of these recommendations include healthcare providers' expertise and patients' understanding of the disease [19].

Interestingly, many respondents rely on diabetic books, often referred to as the "green book," as their primary reference for diabetic foot screening. These books, established in 2000, serve as standard clinic-held records for diabetes management, facilitating continuity of care between different service providers. While the diabetic book includes a checklist for diabetes-related complications, it may not cover all aspects of diabetic foot screening compared to the official Clinical Practice Guidelines (CPG). Furthermore, findings indicate that healthcare providers have not fully adhered to the CPG's checklist for diabetic foot assessment, which includes symptoms and various examinations to stratify risk levels. This finding is similar to a study done



in Scotland, in which they found that none of the general practitioners or nurses interviewed had ever completed the Scottish Care Information-Diabetes Collaboration (SCI-DC) foot risk assessment tool despite diabetic foot risk assessments being a part of the Quality and Outcomes Framework [20].

Additionally, a significant portion of healthcare providers were unaware of the updated version of the CPG, indicating a lack of training and dissemination of information. A study by M. Gershater (2016), supports this and shows a lack of awareness of the existing guidelines among healthcare providers [21]. A study by J. Commons et al.(2018) also highlighted that clinicians might be unaware of the guidelines or sceptical of the value of guidelines because of their lack of evidence base [22]. The CPG of the diabetic foot had been developed to facilitate a continuously updated version of the care process for this vulnerable patient group. The responsibility for organizing teamwork, motivating team members, and facilitating education lies with team leaders, especially considering varying levels of knowledge and resistance to change among team members.

Availability of the CPG is also a concern, with many respondents reporting difficulty accessing it in their clinics, potentially due to budget constraints or lack of awareness. This is corroborated by a study conducted in Western Australia, which revealed that the 2011 National Health and Medical Research Council, Australia guidelines had received very little prior use, the stratification of the intermediate risk category was subpar even after training, and the resources needed to conduct a diabetic foot assessment were not readily accessible in the state's rural and remote health services [23].

## **Conclusion**

This study reveals discrepancies in diabetic foot care practices compared to guidelines, highlighting non-standard workflows and lack of dedicated teams. It urges prioritizing foot care

training and support at all healthcare levels to improve practices, emphasizing the need for management oversight and effective guideline dissemination. Integrating the CPG on the Management of Type 2 Diabetes Mellitus (6th Edition) with the CPG on the Management of Diabetic Foot (2nd Edition) into a single, comprehensive guideline for diabetes management in primary care would enhance accessibility and promote a more holistic approach. This combined CPG would provide healthcare providers with a streamlined reference that addresses both glycaemic control and the prevention and management of diabetic foot complications.

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## **Authors Contribution**

The first two authors are the main contributors to this study in designed, managed and wrote the paper. The remaining authors provided intellectual and technical input to the manuscript for publication.

## **Ethical approval**

Ethics approval to conduct this study was obtained from the local medical research ethics committee (MREC: NMRR-21-1252-60118).

## **Conflict of interest**

The authors declare that they have no conflicts of interest.

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## **Data sharing statement**

Data only available upon request

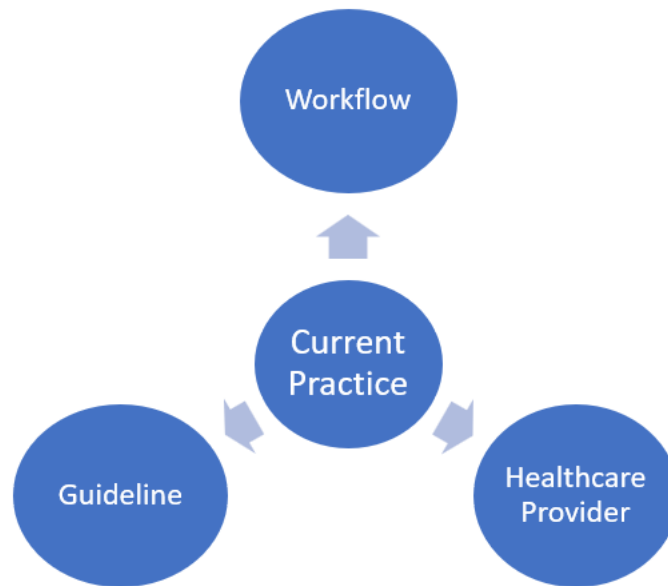


Figure 1. Current structure in managing diabetic foot.

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ORIGINAL ARTICLE

## Practices Contributing to Healthy Longevity among Elderly Population in Ipoh, Perak.

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### Abstract

**Background:** Healthy longevity refers to living a long life while maintaining good health, influenced by genetics, lifestyles, and overall well-being.

**Aim:** This study examines lifestyle, social, and spiritual practices contributing to longevity among elderly individuals aged 80 years and above in Perak.

**Materials and method:** A mixed method study was conducted, involving interviews of 5 elderly individuals aged 85 and above, for the qualitative component, and 55 elderly individuals were subjected to a self-administered questionnaire for the quantitative component. The questionnaires aimed to obtain information on sociodemographic and health information, along with details on lifestyle, social, and spiritual practices.

**Results:** The study found that healthy aging and longevity were associated with proper diet, active living and adequate sleep. Early healthcare interventions helped reduce disease-related morbidity. Social interactions improved mental health, and high spirituality, including feelings of gratitude played a crucial role in well-being. Quantitative data showed 56.4% of respondents ( ) had a normal body mass index (BMI), 61.8% were physically active. Most consumed rice regularly (78.2%), along with meat (89.1%), seafood (92.7%), dairy products (83.7%), vegetables (63.6%). and fruits (>80%). Majority never smoke (70.9%) or drink alcohol (76.4%). However, almost 90% had comorbidities, especially hypertension (78.2%), diabetes mellitus (56.4%) and dyslipidemia (54.5%), with 87.3% on medication and 76.4% showing good disease control. Most respondents had good relationships with relatives and friends (60%) and had moderate to high social engagements (more than 60%). High spirituality was practiced by 72%. Those under 85 had significantly higher social engagements ( $p<0.03$ ), while higher education was significantly associated with high spirituality.

**Conclusion:** Proper diet, active lifestyles, social interactions, spiritual well-being, no smoking, and non-excessive alcohol consumption contribute to healthy aging and longevity.

**Keywords:** *elderly, lifestyles, longevity, social interactions, spiritual well-being.*

## Introduction

The world is currently experiencing a significant increase in the aging population and Malaysia follows a similar trend. Between 2000 and 2050, the proportion of people over 60 is expected to double from 11% to 22%, increasing from 605 million to 2 billion people [1]. In Malaysia, the elderly population (aged 60 and above) is projected to increase from 7.1% in 2020 to 14.5% in 2040 [2]. This growth is driven by increased life expectancy, declining birth rates, and improved healthcare services.

The aging population in Malaysia presents challenges, particularly for the healthcare system, which needs to adapt to cater for the needs of the elderly. Comprehensive policies and programs are required to ensure their well-being. However, the aging process and longevity are not uniform across all populations and are associated with genetics, lifestyle, and overall health. Healthy longevity refers to living in good physical, cognitive and social health throughout the biological life span. To achieve these goals, requires maintaining physical and mental health, following a nutritious diet, avoiding smoking as well as excessive alcohol usage, and staying mentally active to prevent undesired outcomes of ageing [3].

Numerous studies have explored factors influencing health in the elderly and their correlation with longevity. There is evidence suggesting that longevity is influenced largely by genetic and familial background [4] of which certain genes are associated with advanced age [5]. Some studies have found that physical activities were strongly associated with longevity. For example, a study done in Northeastern Thailand found that elderly individuals aged 80 to 105 had normal body mass index (BMI), practiced daily physical activities such as walking for 2 to 3 kilometers per day, arm swinging, Tai Chi, bar slope dancing, gardening, etc. Other lifestyle factors such as a proper diet, good nutrition, cigarette smoking avoidance, controlled alcohol consumption, adequate sleep and rest as well as compliance with medication also play an important role in longevity [6]. Good emotional,

familial, and financial support is vital in leading a stress-free life thus leading to an enhanced life span [7].

Despite growing interest in elderly care there is limited research on their specific needs in Malaysia. Thus, this study aims to study practices among the elderly aged 80 years and above in Perak, that contribute to healthy longevity.

## Materials and methods

This mixed-method study involves interviews for the qualitative, and a self-administered questionnaire for the quantitative component, aimed at identifying practices that contribute to healthy longevity among elderly individuals in Perak. The target population includes Malaysian elderly people aged 80 and above, living in Ipoh and appear healthy. The age cut-off of 80 years was based on Malaysia's life expectancy of approximately 76 years. For the qualitative study, 5 respondents were selected via snowballing method. The quantitative sample size of 55 respondents was calculated based on the expected proportion of 8% of elderly individuals above 80 years old in Kinta District, with a 95% confidence interval. Convenient sampling and snowballing technique were used to recruit participants with referrals from those participants until the targeted sample size was reached.

For the qualitative interviews, eligible respondents provided signed informed consent and the conversations were recorded. The interviews focused on individual practices of respondents contributing to longevity and coping strategies for current living. For the quantitative component, all consented respondents completed a self-filled questionnaire. Variables measured included sociodemographic and health status such as age, gender, race, religion, education, previous occupation, co-morbidities, treatment and compliance with medication, smoking habits and alcohol consumption. Practices such as lifestyles, dietary intake, social and spiritual beliefs were also assessed. The content validity of the questionnaires was evaluated by 2 senior lecturers

in the Royal College of Medicine Perak and pretested with 10 people in the community.

### **Plan for Data Analysis**

The qualitative data from the interviews were transcribed for analysis. All collected data were read several times by the researchers. Constant comparison and verification were done, comparing answers within the same interview and across different interviews of all the participants. The themes were searched, mapped, and interrelated so that the meaning of the themes obtained from the interview could be interpreted. For the quantitative data, the data collected were cleaned and analyzed using SPSS version 26. The social engagements were measured using six questions and were scored (None=0, one=1, two = 2, three to four = 3, five to eight = 4 and nine or more = 5). Score of 0-10 was considered low, 11-20 as moderate and 21-30 as high engagements. The spiritual belief levels were measured using eleven questions and were scored (Never = 0, seldom =1, often = 2, regularly= 3). A score of 0-10 was considered low spirituality, 1-20 as moderate and 21-33 as high spirituality. The frequency and normality tests were performed. The Chi-square test was used to determine the association between some practices and sociodemographic variables. A  $p < 0.05$  was considered significant.

### **Ethical Consideration**

Ethical approval was obtained from the Ethics Committee of Universiti Kuala Lumpur, Royal College of Medicine Perak before conducting this study.

### **Results**

#### **Qualitative data from interview**

Three women and 2 men, aged between 80 and 87 years were interviewed. Four were Malays and one was Chinese. Most had primary education.

#### **Lifestyle practices**

It was found that all respondents had quite similar lifestyles, with their beliefs being the most

important factor in achieving a long and healthy life. One of the respondents, an 85-year-old woman, believed that a good diet and physical activity helped her for a long healthy life.

*“For me the most important things are diet and physical activity. For diet, we can eat everything but need to make sure we take it in small amounts such as sugar and fat. Other than that, physical activity is also important to me. I will do gardening, sweeping and walking to my neighbors’ house. I sweat a lot when I do this kind of activity and makes me feel more energetic. I also do exercise help me to feel stronger to do other things.”*

This study showed that the inclusion of religious and spiritual practices, alongside other health factors, influences longevity. Some of the responses,

*“I believed that maintaining a good lifestyle was the main key factor in keeping longevity such as exercising everyday by involving themselves in certain activities like brisk walk, tai chi and gardening. Next, for the dietary habit, I will control my carbohydrate portion, reduce sweetened food or drinks and avoid junk food. Furthermore, having enough sleep also improve longevity.”*

*“To me, eating habits are the most important. I always ensure adequate plain water consumption and consume fruits and vegetables every day. I also avoid salty foods like ‘budu’ or salted fish. However, I do like eating sweet snacks like ‘kuih’ daily, and I compensate and control the sugar intake by using stevia for my drinks such as tea or coffee. Other than that, I do morning exercise daily by brisk walk about 20 minutes. I also ensure I have good sleep and enough rest every day.*

One of the women stated that physical activity and cautious eating habits could help someone stay healthy as they get older. Additionally, she

mentioned that the most important thing in achieving a long and healthy life is compliance with treatment.

*“People who are physically activity seem to be able to stay healthy when they get older. A lot of people around me who do office work or live sedentary life tend to be less independent when they get older. Some of my old friends who are younger than me can’t even walk by themselves now, but those who worked as cleaner like me or exercise every day mostly are still able to be independent with their daily activities.”*

*“My parents both have multiple diseases, and they were not compliant with the treatment and lifestyle changes. So, I learn from it and be cautious with eating habits. After I was diagnosed with diabetes mellitus and hypertension, I avoided eating store-bought food and cooked my own meal. I ate less carbohydrates and ate a lot of vegetables. I believe that is why my sugar level is always controlled even though it’s been over 30 years since I have diabetes mellitus. But I rarely do exercise before because I already do a lot of physical work during working hours. Now that I have already retired, I just take a walk every morning (10 – 15 minutes a day) or walk to the store to buy groceries. But the most important thing is compliant to treatment. For me, the medication is like a daily food already, and the health checkup also has become a routine.”*

One of the 86-year-old man gave some useful advice,

*“Please stop eating unhealthy foods like fast foods and try to eat more vegetables, fruits and always control your meal portions.”*

*“I will usually do some gardening and house chores like mopping and sweeping. Sometimes I would take a walk around the neighborhood. In my opinion, it is important to move around and do an activity because it will help your brain to keep*

*functioning and prevent your body from getting muscles aches.”*

### **Social practices**

Strong social support from family and friends can help people to have long and healthy life. One of the female respondents mentioned that she always received support from family and friends to cope with life’s stresses and help reduced her risk of developing serious diseases.

*“We need to have friends so we can have someone to talk to. For example, I will call them or go out together if I have free time. So, I can share my problems with my friends. Sometimes we also need to ask them if they have any problems. We need to share our problems with others. If we just hold it without sharing it, it can make us more stressed and can have a bad impact on our health. That’s why it is important to share the problems with friends or family members.”*

Another respondent believed that they felt happier and more motivated to work when they had good interactions with others. She also participated in activities with other people to tighten their relationships.

*“It is very important to have people around us. When we have people to talk to, we are happier, we would have the spirit to do work, but when we are alone, we won’t even have an appetite to eat. After my husband passed away 10 years ago, my son asked me to live with him so he can take care of me. I still meet and talk with my old friends from school who live nearby, and we still talk and chatting about everything like when we were in school.”*

*“I love cooking and watching cooking videos on television and internet. I don’t know if it is related to longevity, but it gives me joy to have something interesting to do every day. I learn a lot of recipes and cook with my family. So, it became our family activity. Luckily, they also like to cook, so we can*

*talk while spending time together, because I think it has become harder for old people like me to talk and chat with the younger generation nowadays. Maybe due to different interest and commitment, but we must find a way so that we can maintain our close relationship,”*

The respondents also believed that maintaining good relationships with people was essential for having a good support system. Keeping closer relationships with family members seemed to be an important recipe for long life. Some of their responses,

*“Personally, I think it’s important that we keep good social relationships with others, as we need good support systems.”*

*“By interacting with family members and relative, give advice and opinion to one closer in order to maintain good relationship.”*

*“I currently don’t have a lot of friends left as they have all passed away. Only a few of them left and I rarely meet them but sometimes I meet them at the mosque after performing our prayers. In terms of family, my child and grandchildren always come and visit me. If they do not have the time to do that, they will at least call and asked if I am doing okay, and we will just talk about our life updates. One of my children has passed away but the others are still healthy, and they visit me regularly. They just came and visit me during ‘raya’ celebration. So, I think that having someone to talk to is very important so that you would not feel lonely, and you have someone to count on especially your children. For me, I still have my wife, so I do have someone to talk to everyday. I can share all my worries to my wife and children.”*

### **Spiritual practices**

All respondents have the same belief that spirituality could influence longevity. Frequent prayers to God helped them find comfort, hope,

and inner peace. A positive and helpful attitude about life and illness could lead to better health outcomes. Some of their quotes,

*“Not easily stressed due to my belief of accepting everything wholeheartedly and feeling of great gratitude would support healthy aging,”*

*“I always participate in tazkirah or religious lecture at mosque. I always performed prayer and fasting to improve my health and improve my self-realization. Furthermore, I will make sure that I will keep good relationships with family members and relative to improve mental wellbeing.”*

*“I believe that the most important thing is to always perform your prayers and do lots of ‘doa’ so that we are blessed with good health and live longer. Life and death are something that we could not plan as it is according to God’s will. I am very thankful for all the fortunes that I currently have right now, and I am still given a chance to be alive with my spouse.”*

*“I am rarely going to church, but I do believe that each of us has purpose in life. Since I was young, I prayed everyday so that my life will become better. Now, even though I am not rich, I am satisfied and content with my life. I believe I finally got what I worked hard and prayed for.”*

## **Quantitative Data from Questionnaire**

### **Sociodemographic data**

In this study, out of 55 respondents, the majority were elderly individuals aged 80-84 years (74.5%), female (58.2%), Malay (50.9%), had a primary school education (40%) and were retired government employees (60%) (Table 1)

### **Health status**

Most respondents were either of normal weight (56.4%) or overweight (23.6%). Almost 90% of the respondents reported having co-morbidities, especially hypertension (78.2%), diabetes mellitus (56.4%), and dyslipidemia (54.5%).



Most of them took medication for their illnesses (87.3%) and have good compliance (76.4%). However, half of them had a disability that limited their activities (Table 2).

### **Lifestyle practices**

Most of them practiced frequent physical activity (61.8%), especially brisk walking (60%). Very few were current smokers (5.5%), with most being either former smokers or never having smoked. Among smokers and former smokers, most smoke less than 10 cigarettes or less daily. Most of them did not consume alcohol. More than 70% of respondents slept between 5 to 8 hours daily (Table 3).

### **Dietary intake practices**

In terms of food intake, most respondents consumed rice (78.2%) and vegetables (63.6%) regularly, and more than often taking flatbread (68.1%), meat (89.1%), fish and sea foods (92.7%), dairy products (83.7%), legumes (54.5%) and fruits (more than 80%). Although 63.7% of respondents seldom or never took salty food, sweet foods seemed to be a choice by more than 60% of them, especially beverages (70.9%) (Table 4).

### **Social relationship practices**

The study found that most respondents have good relationships with their relatives and friends. About 60% were in regular contact with their relatives with at least 3 relatives per month, and around 90% felt at ease and received help from at least one relative. Most respondents continued to get moral support and help from friends. Fewer than 10% had no contact with friends, while more than 50% still had more than 5 friends to chat with. About 45% of respondents still had close friends who could offer help in times of difficulties (Table 5).

### **Social Engagements level of Respondents**

The prevalence of low social engagement was 36.4%, moderate 34.5% and high 29.1%. For the association analysis, moderate and high social

engagement were combined. The age was reclassified into less than 85 and more than 85 years old, ethnic groups into Malays and non-Malays and education levels into informal/primary and above. It was found that age was significantly associated with social engagement ( $p = 0.03$ ) of which younger participants seemed to be more socially engaged (70.7%) compared to the older participants (42.9%). Other sociodemographic factors showed no significant relationship with the level of social engagement.

### **Spiritual belief practices**

A strong spiritual belief seemed to be very well related to healthy aging and longevity. The study showed that most respondents prayed regularly (63.6%), more often self-reflected on the purpose of life (71%), experienced self-realization (72.8%), helped others (68.2%), considered the needs of others (76.4%) and felt great gratitude (81.8%) (Table 7).

### **Spiritual belief levels of the respondents**

The prevalence of low spirituality was 3.6%, moderate 30.9% and high 65.5%. For the association analysis, low and moderate spirituality was combined. The age was reclassified into less than 85 years and 85 years or more, ethnic groups into Malays and non-Malays, and education levels into informal/primary and above. It was found that spirituality and belief were not associated with age, gender or ethnic groups. However, the level of education was significantly associated with spirituality and belief. High spirituality and belief were common among elderly with secondary education and higher compared to those with informal or primary education ( $p$ -value  $< 0.05$ ) (Table 8).

### **Discussion**

Analysis of qualitative data showed insights into the perspectives of elderly individuals, aged 80 to 87, regarding factors contributing to longevity. The study further explores lifestyle, social, and

spiritual practices as crucial elements influencing health outcomes in the elderly population.

All respondents unanimously agreed that lifestyle factors such as diet, physical activity, and adherence to treatment regimens play a huge role in maintaining long-term health. The emphasis on dietary habits, including portion control, balanced nutrition, and hydration, underscores the significance of mindful eating practices in disease management and prevention. Additionally, engaging in regular physical activity, whether through structured exercise routines or daily chores, reflects a commitment to maintaining mobility and vitality in later life. These findings align with global research, where lifestyle modifications such as dietary habits and physical activity have been shown to extend life expectancy [8-9]. Notably, the incorporation of lifestyle modifications following health diagnoses highlights the adaptive capacity of individuals to proactively manage their well-being.

In terms of social, the significance of social connections emerges as a key theme in the narratives, with respondents attributing their well-being to the support received from family and friends. Interactions with peers serve as platforms for emotional release, stress management, and friendship, thereby bolstering mental resilience and overall satisfaction with life. The role of familial relationships, particularly in providing care and companionship, highlights the importance of maintaining social bonds in mitigating loneliness and fostering a sense of belonging in older adults. Moreover, the reciprocity of support within familial networks exemplifies the symbiotic nature of intergenerational relationships in promoting holistic health outcomes. This has been shown in a few studies that discuss the impact that social involvement plays in extending life expectancy in the elderly [10-11].

Spirituality plays a fundamental role in coping strategies and meaning making for the respondents. Religious rituals, prayer, and seeking solace in faith communities offer ways to

find inner peace, acceptance, and gratitude amidst life's challenges. The narratives demonstrate how spiritual beliefs serve as sources of resilience, influencing attitudes towards illness, mortality, and life satisfaction. Spirituality helps promote psychological well-being and adaptive coping mechanisms in the face of adversity by cultivating a sense of purpose and transcendence. While some may see the inclusion of religion alongside other health factors as unscientific, multiple studies conducted globally have shown that spiritual influences seem to increase with age [12]. The qualitative part of the study highlights the complex interactions between lifestyle choices, social support systems, and spiritual beliefs in shaping health outcomes among elderly. By recognizing and addressing the diverse needs and perspectives of older individuals, healthcare professionals can design comprehensive interventions that promote holistic well-being and facilitate successful aging. The quantitative components on the other hand, provide the magnitude and extent of the factors perceived or felt by the respondents. It was found that almost 90% of the study respondents had already established the state of disease, especially hypertension, diabetes mellitus, and dyslipidemia. Most of them have been in treatment for many years, strictly complied with the regime, and had a well-controlled disease status. It is well known that mortality is increased by increasing age largely due to ischemic heart disease [13-14].

In this study, most respondents have a normal BMI (56.4%). This corresponds to the high frequency of physical exercise (61.8%) among them. The study showed that most of the respondents were actively involved in physical activity like gardening (40%), jogging (14.5%) and aerobics (12.7%). Most of them never smoke cigarettes (70.9%) or drink alcohol 76.4%). It is widely recognized that regular physical activity can enhance physical and mental functions, as well as reverse the effects of chronic diseases, which can help keep older individuals mobile and independent [15]. Regular physical activity also is safe for healthy and frail older people. It may

reduce the risks of developing cardiovascular diseases or its complications, obesity, muscular weakness (sarcopenia) and falls, depressive disorder, dementia etc. However, many studies showed that participation in physical activities at this age remains low, especially after they retire from work. People aged >80 years are over 50 % less likely than those in their early 50s to engage in sports or to want to increase their activity levels. The level will further decline as the person ages [16].

The dietary habits among respondents seem to be related to their long lifespan. Most of the respondents in the study consume a good and balanced diet. Their intake of carbohydrates, proteins, fats, vegetables, and fruits was according to the recommended portion that has been suggested by the doctors with little to no added sugar, saturated fats, and salt. Portion control is very crucial to prevent overeating and weight gain as the metabolic rate will decrease as age increases. Older people need a lower energy intake, higher protein content to preserve muscle mass, and a greater supply of vitamins and minerals to maintain good bone health. A cross-sectional study among people above 60 years old residing in the Spanish Mediterranean using a food frequency questionnaire found that the compliance to a recommended dietary intake (RDI) was low. However, among those people with higher degree of compliance to the recommended intakes, they have better control of their blood pressure, cholesterol, and glucose levels [17]. Poor compliance with RDI in elderly seemed to be related to decreased appetite, swallowing, and mastication difficulties, which reduce their motivation to eat [18]. A diet that is specifically formulated and textured for the elderly can enhance their quality of life and longevity.

Regarding the social aspects, most respondents frequently met or were in contact with nine or more of their relatives in a month (27.3%). It is well known that family bonding is very important for a sense of purpose and belonging in life. It offers an opportunity for the elderly to connect

with others, especially family members and relatives, that have similar interests and values. They also feel at ease talking about private matters and even ask for help when needed. They believed that maintaining good relationships with close companions would help them with emotional support, reduce the feelings of loneliness, and promote overall well-being [19]. However, a study showed that their social engagement with the community was low. This is probably due to the inability of elderly individuals to use gadgets for connecting with friends and the community. Strong social support has been proven to be one of the main components that can help people stay healthy and have long life. Persistent social interaction can help the elderly to increase mental stimulation, emotional resilience and quality of life [20].

On spiritual and belief factors, most respondents privately pray regularly with 63.6% believing that engaging with religious belief can provide a sense of spiritual nourishment and coping mechanism during times of adversity [20]. They also work in the mind-body discipline, reflect upon the meaning of life, and get to realize the insight as this is one of the ways to maintain healthy longevity. Furthermore, most of them in this study regularly have feelings of great gratitude as having the opportunity to live longer than others (63.6%), similar to findings in another study [21]. A high level of spirituality among respondents (65.5%) in this study was also identified as a key factor in maintaining longevity and staying healthy [22].

The findings from this mixed study support the need for active lifestyle practices, proper dietary intakes and maintaining mental health through social engagements and high spirituality to achieve healthy longevity. However, the results of this study should be interpreted cautiously as it is a cross-sectional and descriptive study, using a self-administered questionnaire and a convenience sampling method. Nevertheless, the results may provide insights into common practices adopted by elderly people in Perak and possibly Malaysia in general to stay healthy in old

age. Larger study with a greater number of samples are highly recommended.

### Conclusion

This study demonstrated that maintaining physical and mental health by practicing proper dietary intakes, active lifestyles, avoiding smoking and excessive alcohol usage, as well as by enhancing social and spiritual health can help to prevent the undesired outcome of ageing and preserve longevity.

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### Conflict of interest

The authors verified that there were no financial or commercial ties that might be viewed as having a potential conflict of interest.

### Authors contribution

The first five authors designed, managed, and wrote the paper as main contributors of this study. The sixth author provided intellectual, technical input and editing of the manuscript for publication purposes.

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None

Table 1. Sociodemographic data distribution among respondents (n = 55)

Variable		Frequency (N)	Percentage (%)
Age	80-84 years old	41	74.6
	85-90 years old	13	23.6
	91-95 years old	1	1.8
Gender	Male	23	41.8
	Female	32	58.2
Race	Malay	28	50.9
	Chinese	22	40
	Indian	4	7.3
	Punjabi	1	1.8
Religion	Islam	28	50.9
	Christian	13	23.6
	Buddhism	10	18.2
	Hinduism	3	5.5
	Sikh	1	1.8
Education level	No formal education	3	5.5
	Primary	22	40
	Secondary	15	27.3
	Tertiary	15	27.3
Previous occupation	Government	33	60
	Private	10	18.2
	Self-employed	1	1.8
	Housewife	11	20

Table 2. Health status and coping with illnesses of the respondents

<b>Variable</b>		<b>Frequency (N)</b>	<b>Percentage (%)</b>
<b>Body Mass Index (BMI)</b>	Underweight	7	12.7
	Normal	31	56.4
	Overweight	13	23.6
	Obese	4	7.3
<b>Medical Illness</b>	Diabetes Mellitus (DM)	31	56.4
	Hypertension (HPT)	43	78.2
	Ischemic Heart Disease (IHD)	5	9.1
	Hyperlipidemia (HLD)	30	54.5
	Gout	2	3.6
	Bronchial Asthma (BA)	1	1.8
	Parkinsonism (PD)	1	1.8
	Liver cirrhosis (LC)	1	1.8
	Glaucoma	1	1.8
	No co-morbidity	6	10.9
	<b>Treatment of Illness</b>	Yes	48
No		1	1.8
Not applicable		6	10.9
<b>Compliance to Medication</b>	Yes	42	76.4
	No	4	7.2
	Partially	3	5.5
	Not applicable	6	10.9
<b>Limitation of Activities due to Illness</b>	Yes	28	50.9
	No	21	38.2

Table 3. Lifestyles factors

<b>Variable</b>		<b>Frequency (N)</b>	<b>Percentage (%)</b>
<b>Body Mass Index (BMI)</b>	Underweight	7	12.7
	Normal	31	56.4
	Overweight	13	23.6
	Obese	4	7.3
<b>Frequency of physical exercise</b>	Frequent	34	61.8
	Rarely	18	32.7
	Never	3	5.5
<b>Type of physical activity</b>	Brisk walking	33	60
	Jogging	8	14.5
	Tai Chi	6	10.9
	Aerobics	7	12.7
	Gardening	22	40
	Cycling	1	1.8
	Physiotherapy	2	3.6
	House chores	4	7.3
	None	4	7.3
<b>History of smoking</b>	Currently	3	5.5
	In the past	13	23.6
	Never	39	70.9
<b>Smoking habit</b>	10 or less daily	10	18.1
	11-20 daily	4	7.3
	21-30 daily	1	1.8
	31 or more	1	1.8
	Not applicable	39	70.9
<b>Alcohol consumption</b>	Frequent	1	1.8
	Rarely	12	21.8
	Never	42	76.4
<b>Sleep and rest</b>	Less than 5 hours	9	16.4
	5-6 hours	19	34.5
	7-8 hours	21	38.2
	More than 8 hours	6	10.9

Table 4. Dietary intake.

<b>Variable</b>		<b>Frequency (N)</b>	<b>Percentage (%)</b>
<b>Dietary habit: Rice and noodles</b>	Regularly	43	78.2
	Often	9	16.4
	Seldomly	2	3.6
	Never	1	1.8
<b>Dietary habit: Oats</b>	Regularly	3	5.5
	Often	9	16.4
	Seldomly	11	20.0
	Never	32	58.2
<b>Dietary habit: Flatbread</b>	Regularly	7	12.7
	Often	31	56.4
	Seldomly	13	23.6
	Never	4	7.3
<b>Dietary habit: Meats</b>	Regularly	23	41.8
	Often	26	47.3
	Seldomly	2	3.6
	Never	4	7.3
<b>Dietary habit: Fish and seafood</b>	Regularly	18	32.7
	Often	33	60.0
	Seldomly	2	3.6
	Never	2	3.6
<b>Dietary habit: Dairy</b>	Regularly	14	25.5
	Often	32	58.2
	Seldomly	7	12.7
	Never	2	3.6
<b>Dietary habit: Legumes</b>	Regularly	8	14.5
	Often	22	40.0
	Seldomly	15	27.3
	Never	10	18.2
<b>Dietary habit: Tropical fruits</b>	Regularly	19	34.5
	Often	33	60.0
	Seldomly	3	5.5
	Never	0	0
<b>Dietary habit: Fruits</b>	Regularly	11	20.0
	Often	34	61.8
	Seldomly	10	18.2
	Never	0	0
<b>Dietary habit: Vegetables</b>	Regularly	35	63.6
	Often	18	32.7
	Seldomly	1	1.8
	Never	1	1.8
<b>Dietary habit: Local snacks</b>	Regularly	7	12.7
	Often	30	54.5
	Seldomly	11	20.0

	Never	7	12.7
<b>Dietary habit: High salt foods</b>	Regularly	1	1.8
	Often	19	34.5
	Seldomly	15	27.3
	Never	20	36.4
<b>Dietary habit: Sweet foods</b>	Regularly	3	5.5
	Often	34	61.8
	Seldomly	7	12.7
	Never	11	20.0
<b>Dietary habit: Beverages</b>	Regularly	10	18.2
	Often	29	52.7
	Seldomly	11	20.0
	Never	5	9.1



Table 5. Social engagement factors

<b>Variable</b>		<b>Frequency (N)</b>	<b>Percentage (%)</b>
<b>How many of your relatives do you see or hear from at least once a month?</b>	None	2	3.6
	One	10	18.2
	Two	10	18.2
	Three to four	8	14.5
	Five to eight	10	18.2
	Nine or more	15	27.3
<b>How many relatives do you feel at ease with that you can talk about private matters?</b>	None	6	10.9
	One	14	25.5
	Two	11	20.0
	Three to four	5	9.1
	Five to eight	13	23.6
	Nine or more	6	10.9
<b>How many relatives do you feel close to such that you could call on them for help?</b>	None	4	7.3
	One	8	14.5
	Two	15	27.3
	Three to four	7	12.7
	Five to eight	13	23.6
	Nine or more	8	14.5
<b>How many of your friends do you see or hear from at least once a month?</b>	None	5	9.1
	One	12	21.8
	Two	8	14.5
	Three to four	5	9.1
	Five to eight	11	20.0
	Nine or more	14	25.5
<b>How many friends do you feel at ease with that you can talk about private matters?</b>	None	15	27.3
	One	9	16.4
	Two	10	18.2
	Three to four	9	16.4
	Five to eight	5	9.1
	Nine or more	7	12.7
<b>How many friends do you feel close to such that you could call on them for help?</b>	None	11	20.0
	One	13	23.6
	Two	12	21.8
	Three to four	6	10.9
	Five to eight	4	7.3
	Nine or more	9	16.4

Table 6. Association between social engagements and sociodemographic factors.

<b>Social Factors</b>	<b>category</b>	<b>n</b>	<b>Low Social Engagement</b>	<b>Moderate/high Social Engagement</b>	<b>P-value*</b>
<b>Age</b>	<85	41	12 (29.3%)	29 (70.7%)	0.03
	≥85 (n=14)	14	8 (57.1%)	6 (42.9%)	
<b>Gender</b>	Male	23	11	12	0.13
	Female	32	9	23	
<b>Ethnic group</b>	Malays	28	8	20	0.22
	Non-Malays	27	12	15	
<b>Education</b>	Informal or primary	25	9	16	0.95
	Secondary and above	30	11	19	

P<0.05 is significant.

Table 7. Spiritual belief factors

<b>Variable</b>		<b>Frequency (N)</b>	<b>Percentage (%)</b>
<b>I privately pray</b>	Regularly	35	63.6
	Often	18	32.7
	Seldom	0	0
	Never	2	3.6
<b>I go to mosque/ church / temples etc.</b>	Regularly	15	27.3
	Often	13	23.6
	Seldom	21	38.2
	Never	6	10.9
<b>I work on a mind-body discipline</b>	Regularly	8	14.5
	Often	9	16.4
	Seldom	14	25.5
	Never	24	43.6
<b>I reflect upon meaning and purpose of life</b>	Regularly	14	25.5
	Often	25	45.5
	Seldom	15	27.3
	Never	1	1.8
<b>I try to get insight or self-realization</b>	Regularly	14	25.5
	Often	26	47.3
	Seldom	13	23.6
	Never	2	3.6
<b>In my private area, religious symbols are important to me</b>	Regularly	22	40.0
	Often	21	38.2
	Seldom	2	3.6
	Never	10	18.2
<b>I participate in religious events</b>	Regularly	15	27.3
	Often	9	16.4
	Seldom	19	34.5
	Never	12	21.8
<b>I help others</b>	Regularly	27	49.1
	Often	16	29.1
	Seldom	9	16.4
	Never	3	5.5
<b>I consider the needs of others</b>	Regularly	26	47.3
	Often	20	36.4
	Seldom	7	12.7
	Never	2	3.6
<b>I have a feeling of great gratitude</b>	Regularly	35	63.6
	Often	17	30.9
	Seldom	3	5.5
	Never	0	0
<b>I still have learned to experience and value beauty</b>	Regularly	29	52.7
	Often	16	29.1
	Seldom	9	16.4
	Never	1	1.8

Table 8. Association between spirituality and belief with socio-economic factors.

<b>Spirituality Factors</b>	<b>category</b>	<b>n</b>	<b>Low/moderate spirituality</b>	<b>high spirituality</b>	<b>P-value*</b>
<b>Age</b>	<85	41	14	27	0.92
	≥85	14	5	9	
<b>Gender</b>	Male	23	10	13	0.17
	Female	32	8	23	
<b>Ethnic group</b>	Malays	28	7	21	0.13
	Non- Malays	27	12	15	
<b>Education</b>	Informal or primary	25	17 (68.0%)	8 (32.0%)	0.00002 (S)
	Secondary and above	30	2 (6.7%)	28 (93.3%)	

P<0.05 is significant.

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ORIGINAL ARTICLE

## The Malay Version of Dialysis Specific Nutrition Literacy Scale (DSNLS): Translation and Face Validation.

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### Abstract

**Background:** Nutrition literacy is essential for understanding, assessing, and utilising nutritional information, particularly in end-stage renal disease (ESRD) patients, to improve their overall well-being. A suitable tool is necessary to assess their level of nutrition literacy. Unfortunately, there is a lack of a specific nutrition literacy scale in the Malay language for the hemodialysis patients' population. Therefore, the aim of this study is to translate and validate the Dialysis-Specific Nutrition Literacy Scale (DSNLS) into Malay. **Methods:** The questionnaire was forward and backward translated by experts in dietetics and linguistics following established guidelines, resulting in a pre-harmonised Malay translation and two backward English translations. An expert committee reviewed and harmonised these translated questions. The questionnaire was then evaluated through a cognitive interview and face validity assessment among 15 hemodialysis patients. **Results:** During the expert committee assessment, several phrases underwent revisions to improve clarity and comprehension. Respondents preferred that certain terms be replaced during pre-testing with common phrases used in nutrition education. All questions achieved a face validity index value greater than the acceptable value, indicating good validity ( $\geq 0.87$ ). **Conclusion:** The Malay version of the DSNLS underwent translation and face validation, and the findings demonstrate that it is clear and comprehensible for evaluating nutrition literacy among local hemodialysis patients.

**Keywords:** *Dialysis, DSNLS, End-stage renal disease, Malay, Nutrition literacy, Translation.*

## Introduction

The World Health Organization (WHO) stated that health literacy is the literacy that entails people's knowledge, motivation, and competences to access, understand, appraise, and apply health information in order to make judgements and take decisions in everyday life concerning health care, disease prevention, and health promotion to maintain or improve quality of life during the life course [1]. Poor health literacy can contribute to poor health consequences [1,2], particularly impacting minority communities, elderly individuals, and individuals with lower levels of education and/or income [2]. Meanwhile, nutrition literacy refers to 'the degree to which individuals have the capacity to obtain, process, and understand nutrition information and skills needed in order to make appropriate nutrition decisions' [3]. The process involves acquiring knowledge of nutritional principles and possessing the ability to perceive, assess, and utilize nutritional information, specifically to be cognizant of the various nutrients and their influence on overall well-being.

In Malaysia, the incidence of patients with end-stage renal disease (ESRD) necessitating renal replacement therapies, such as hemodialysis, has exhibited a consistent upward trajectory over the course of the last two decades. According to the 30<sup>th</sup> Report of the Malaysian Dialysis and Transplant Registry, there were a total of 9,123 new dialysis patients, consisting of 7,477 new patients receiving hemodialysis and 1,646 new patients receiving peritoneal dialysis in 2021 [4]. Kidney dysfunction among ESRD patients who are undergoing dialysis treatment requires specific dietary guidelines to prevent possible health outcomes such as fluid overload, electrolyte imbalance, and protein-energy depletion.

Dietary adherence among hemodialysis patients has been found to be reasonably predicted by nutrition literacy [5]. A previous cross-sectional study conducted in Malaysia involving 218

hemodialysis patients revealed that almost half (46.3%) of the patients exhibited a limited level of nutrition literacy based on the Dialysis Specific Nutrition Literacy Scale (DSNLS) [5]. It was found that low nutrition literacy is common among patients who are older, have a lower education level, have shorter dialysis vintage, and have never received nutritional counselling from healthcare professionals [5]. Moreover, lower health literacy among hemodialysis patients has been associated with non-adherence to dialysis treatment, frequent hospitalizations, and increased visits to the emergency department [6]. Therefore, adequate nutrition literacy is crucial to enable them to comprehend, evaluate, and apply diet-related knowledge in their everyday lives. This is essential to improving patients' nutritional management and enhancing their overall well-being.

Instruments for health literacy assessment designed specifically for dialysis patients are scarce; therefore, previous research has employed general health literacy assessment tools for use among this population. These include the European Health Literacy Survey (HLS-EU-Q) [7], the Health Literacy for Iranian Adults (HELIA) [8], and the Health Literacy Questionnaire (HLQ) [9], which measure functional, interactive, and critical domains of health literacy. More recently, researchers in Taiwan have developed and validated a dialysis-specific health literacy questionnaire among their HD population, which also assesses the three domains [10]. However, there were no scales for nutrition literacy assessment among the dialysis populations, hence the development of the DSNLS [5]. The other nutrition literacy questionnaire for this population was the Nutrition Literacy Evaluation Scale [11] which focused on four dimensions: 'nutrition knowledge level', 'cognitive attitude', 'behavioural practice', and 'information acquisition ability'. Nevertheless, this scale has only been used in China.



Therefore, this study intends to translate and validate the English DSNLS into Malay, as it is the only nutrition literacy questionnaire intended for dialysis patients that has been previously used locally [5]. Researchers and healthcare providers in Malaysia would benefit from the validated Malay version of the DSNLS, through which they may evaluate the nutrition literacy level of the ESRD patients and provide interventions as necessary. This, in turn, could lead to improved nutritional management and an enhanced ability to manage their condition, prevent complications, and maintain overall health among the dialysis patients.

## Materials and methods

### Dialysis Specific Nutrition Literacy Scale (DSNLS)

Based on the HLS-EU-Q [12] that has been validated and utilized in six Asian countries, including Malaysia, the DSNLS was constructed to assess a patient's perceived ability in obtaining (interactive literacy), understanding (functional literacy), appraising, and applying (critical literacy) dietary knowledge pertaining to dialysis [5]. The tool comprises eight items that are graded on a scale of 1 (poor ability) to 3 (good ability). The nutrition literacy mean score is converted into continuous indices using the formula:  $\text{Index} = (\text{Mean} - 1) \times (100/2)$ , which spans from 0 to 100. A higher index indicates a higher level of nutrition literacy where a limited nutrition literacy level has a mean index score of 50 or less, while an acceptable level has more than 50. The DSNLS demonstrated a good content validity (S-CVI/Ave = 0.96) as evaluated by six renal healthcare specialists [5]. Additionally, the entire DSNLS index correlated well with the HLS-EU-Q16 (intra-class coefficients, ICC = 0.792), while the internal consistency (Cronbach's alpha = 0.916) also showed good reliability.

### Study Design

This study employed translational research to translate and validate an English questionnaire

into Malay using the WHO instrument translation guideline [13]. This is to bridge the gap between theoretical knowledge and practical application to ensure that this questionnaire is culturally and linguistically appropriate to be used among the Malay-speaking population.

### Phase 1: Questionnaire Translation

The DSNLS underwent five steps of the translation process: i) forward translation of the questionnaire from English to Malay; ii) synthesis of the pre-harmonized Malay version of the questionnaire; iii) backward translation of the pre-harmonised Malay version of the questionnaire back into English; iv) expert committee review of the translated versions of the questionnaire to develop a harmonised version; and v) pre-testing of the questionnaire (using cognitive interview) among 15 patients who were undergoing hemodialysis procedure at the Pusat Dialisis Majlis Ugama Islam dan Adat Resam Melayu Pahang (MUIP). Each process is described in detail elsewhere [14].

### Phase 2: Questionnaire Validation

Face validity was conducted concurrently with the cognitive interview among the 15 hemodialysis patients [13,15]. The comprehensibility and clarity of each item were evaluated with a four-point Likert scale that encompassed responses ranging from 'Item is not clear and understandable' to 'Item is very clear and understandable'. Items that obtained a face validation index (FVI) value of 0.80 or higher were maintained [15]. Item FVI (I-FVI, percentage of raters who give an item or question a 3 or 4 for clarity and understanding) and scale FVI (S-FVI, mean of the I-FVI scores for all items or the average of all raters' clarity and comprehension) were determined. Items rated with 3 or 4 were recoded as 1, indicating they were clear and comprehensible, while items rated with 1 or 2 were recoded as 0, indicating they were unclear and incomprehensible. An average evaluation from each rater determined the proportion of clarity and comprehension. When

raters agree 100%, the Universal Agreement (UA) score is 1. If not, the UA score would be 0 [15].

This study did not include content validation as the questionnaire items did not possess any subject domains that needed to be addressed [16]. Additionally, the answer section only required respondents to rate their ability in nutrition literacy as either 'poor', 'fair', or 'good'.

### **Ethical Approval**

Approvals to conduct the research were obtained from the International Islamic University Malaysia Research Ethics Committee (Reference No.: IIUM/504/14/11/2/IREC2024-001) as well as the Pusat Dialisis MUIP. The participants provided their informed consent prior to their involvement in the study.

### **Data Analysis**

Numerical data were presented using descriptive analyses such as means and standard deviations, while categorical data were described using absolute numbers and percentages. The descriptive analyses and tabulation of the FVI were conducted using Microsoft Excel.

## **Results**

### **Phase 1: Translation**

During the expert committee review, a few phrases in the pre-harmonised Malay version of the DSNLS (i.e., '*potasium*' and '*sodium*') were suggested to be substituted with Malay words that originate from Latin terms (i.e., "*kalium*" and "*natrium*"). Furthermore, in Item No. 6, the phrase "*menentukan*" was replaced by "*mengenal pasti*" and the sentence was also harmonised to achieve a more precise meaning.

During pre-testing of the questionnaire, most of the respondents commented that they were more familiar with the terms "*potasium*", "*sodium*" and "*fosfat*" instead of "*kalium*", "*natrium*" and "*fosforus*", respectively. This was because these terms were more commonly used in nutrition

education related to kidney disease. In addition, those terms are also proper Malay terms that are loanwords from the English language [17]. As a result, the researchers unanimously agreed to use the terms suggested by the respondents to ensure optimal clarity and better understanding of the questionnaire in the final harmonised Malay version of DSNLS.

### **Phase 2: Face Validation**

The participants of the assessment included five men (33.3%) and 10 women (66.7%), aged from 24 to 72 years. All participants were Malay and had been undergoing dialysis for a range of six months to 22 years, with an average of  $50.5 \pm 60.9$  months.

Five out of eight items were rated as clear and comprehensible by all the respondents (Table 1). Nevertheless, while Items No. 2, 5, and 6 were not rated as clear and comprehensible by all respondents, they were still included in the questionnaire since the FVI values exceeded the acceptable threshold ( $\geq 0.87$ ). Based on these findings, the Malay translation of the DSNLS was deemed as a valid instrument to assess nutrition literacy levels among Malaysian hemodialysis patients.

## **Discussion**

Nutrition literacy among hemodialysis patients is important for improving dietary adherence, providing education and support, and encouraging patient empowerment. Not only does nutrition literacy correlate with nutritional status, but it also significantly influences the quality of life. The patients' level of nutrition literacy will influence their choices of nutrients and eating habits, ultimately influencing the progression of kidney disease [11]. Assessing the level of nutrition literacy can serve as a preliminary evaluation of the patient's knowledge and comprehension of their illness with respect to dietary management and their capacity to make the best decisions for their care.

The current research involved five distinct steps in the process of translating the DSNLS into the Malay language, and face validation was conducted along with pre-testing of the translated questionnaire. Throughout the process of translation, several modifications were made to ensure that specific phrases accurately conveyed the intended meaning as initially used in the English version. The use of accurate terms is emphasised in accordance with the content of nutrition education that is often utilised among the public and patients in Malaysia [18,19]. In addition, feedback and comments provided by participants during the cognitive interview were duly reviewed to improve understanding and avoid any ambiguity in the translated material.

Instead of merely focusing on linguistic or literal equivalency, instruments must be translated and adapted to meet established criteria and ensure cultural and conceptual appropriateness [20]. The cultural equivalency of a translated instrument is how relevant and usable a word, idea, scale, or normative structure is to cultural groups other than the one from which it came [21]. The five dimensions of stepwise validation for cross-cultural equivalence include content, semantic, technical, criterion, and conceptual, which are mutually exclusive [22]. An instrument may be cross-culturally equivalent in one or more dimensions but not in others [22,23]. This study examined the content, semantic, and conceptual equivalence of the translated questionnaire through forward and backward translations and expert committee review. It was important that the questionnaire accurately assessed the same theoretical constructs and maintained their meaning in the culture of interest. The literal meaning of the sentence remains unchanged from the original English version, while preserving its context. As a result, translation into Malay did not give rise to any substantial apprehension regarding cultural equivalence. Furthermore, the good FVI values indicated that the Malay version of the DSNLS is clear and comprehensible.

In line with the findings from the study on the development of the other Malay language health literacy questionnaire [24], which reported acceptable S-FVI/Ave values for all domains, our study also demonstrated high S-FVI/Ave values exceeding the accepted threshold. This consistency underscores the robustness and clarity of our questionnaire items, affirming their face validity.

To the best of the authors' knowledge, this translated version of the DSNLS questionnaire is the only questionnaire assessing nutrition literacy among hemodialysis patients in Malay. This is the first study of its kind to produce a Malay version of the scale and have it validated among local dialysis patients. However, it is demographically typical in that these only comprised Malay respondents because the sampling area was confined to an east-coast state in Peninsular Malaysia, even though purposive sampling was used in the sampling method. Regardless, the availability of this assessment tool in a translated version makes it more convenient and wide-reaching for healthcare professionals in Malaysia to determine patient gaps in nutrition knowledge and literacy, enabling them to provide the necessary education, thus improving health literacy in the country.

## **Conclusion**

The Malay version of DSLNS has undergone the translation and face validation processes according to established guidelines. The findings demonstrated good FVI values, indicating its comprehensibility and clarity among the target respondents. This indicates that the Malay DSLNS can be utilized to assess nutrition literacy levels among hemodialysis patients in Malaysia.

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**Conflict of interest**

The authors declare no conflict of interest.

**Authors' contributions**

WAMAB, RMY, NS, and NAMS came out with the research concept and design. SMK conducted data collection, ran the analysis, and prepared the manuscript. SMK, WAMAB, RMY, and NAMS were involved during the expert committee review. RMY and NAMS provided advice on data analysis and interpretation of the results and reviewed the manuscript. The manuscript has been approved for publication by all authors.

Table 1. Face validity index for the Malay version of DSNLS (N=15)

Items	Questions							
	1	2	3	4	5	6	7	8
S-FVI/Ave	1.00	0.87	1.00	1.00	0.87	0.93	1.00	1.00
S-FVI/UA	1.00	0	1.00	1.00	0	0	1.00	1.00
Proportion Clarity/Comprehensible	1.00	0.87	1.00	1.00	0.87	0.93	1.00	1.00

S-FVI/Ave: Scale-level face validity index based on the average method; S-FVI/UA: Scale-level face validity index based on the universal agreement method

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ORIGINAL ARTICLE

## Reliability and Construct Validity of Maqasid Shariah Knowledge Questionnaire.

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### Abstract

Maqasid Shariah is a holistic view of life with the concept of maintaining good conditions and ensuring the welfare of all humankind while preventing any harmful act, evil, or injury. In the context of medical practice, it shares similar objectives, particularly in promoting well-being and upholding principles of harm reduction. However, no local questionnaire or assessment tool currently available to measure the level of understanding of our community regarding Maqasid Shariah principles. Therefore, a newly developed, reliable, and valid questionnaire is needed. This study aimed to develop and validate a newly created questionnaire to assess knowledge of Maqasid Shariah. The questionnaire consists of 20 items, including a mix of true and false-choice questions and one best answer question on Maqasid Shariah topics. The questionnaire was distributed to 254 members of the medical community. Reliability was determined using Cronbach's alpha for internal consistency, while construct validity was assessed using factor analysis. An acceptable degree of internal consistency was observed for the final 12 items (Cronbach's alpha = 0.700). Factor analysis showed two meaningful components, which represent the background domain and the knowledge domain. This study indicates that this questionnaire is a reliable and valid tool for assessing the knowledge of respondents about Maqasid Shariah.

**Keywords:** *Maqasid Shariah, questionnaire, validation.*



## Introduction

*Maqasid Shariah* is one of the fundamental principles of human life. Its objectives are to bring benefit to mankind in both this world and the hereafter, while preventing corruption and evil [1]. *Maqasid* literally means "straight path" and "justice." Overall, *Maqasid Shariah* can be regarded as the meanings and wisdom considered by the lawgiver in all or most circumstances of Shariah. This consideration is not confined to specific types of Shariah rulings but governs all aspects of life. There are three types of rules under Shariah: rules relating to belief (Aqidah), rules relating to morality (Akhlak), and rules relating to acts of subjects or practical rules (Fiqh). The essential *Maqasids* are concerned with the protection and preservation of five fundamental values: religion (al-din), life (al-nafs), intellect (al-'aql), progeny (al-nasl), and wealth (al-mal) [2].

The primary objectives (*Maqasid*) typically include the preservation of faith (din), life (nafs), intellect ('aql), lineage (nasl), and property (mal) [1-3]. Based on the priority of saving lives, *Maqasid Shariah* places great importance on preserving life, encouraging the development of medical practices and interventions that save lives and improve health. It promotes the advancement of medical research and technology to prevent, diagnose, and treat diseases effectively. Regarding the preservation of intellect, *Maqasid Shariah* acknowledges the importance of mental health alongside physical health, advocating for comprehensive care that includes psychological well-being. In the preservation of faith, *Maqasid Shariah* promotes a holistic approach to medicine that considers spiritual well-being, ensuring that care aligns with patients' religious and spiritual beliefs. It also encourages responsible reproductive health practices and the protection of family lineage through ethical medical interventions in fertility, prenatal, and postnatal care, thus preserving lineage. Finally, in the preservation of property, *Maqasid Shariah* advocates for affordable healthcare services, ensuring that financial constraints do not hinder access to essential medical care [1-3].

Given that the preservation of the five essential *Maqasids* aligns with medical ethics and practices, this concept has long been incorporated into medical issues and guidelines, particularly among Muslim practitioners and patients. In medical practice, *Maqasid Shariah* shares similar objectives and aims in promoting well-being and upholding harm reduction principles [3,4]. The intersection of both can be seen as an integration of the theoretical framework governed by *Maqasid Shariah* and the practical aspects delivered by healthcare providers in medical settings. Early Muslim physicians, notably Ibn Sina and Al-Razi, demonstrated Islamic principles in medical and healthcare practices since the early history of medicine [3,4]. This integration has been emphasized in medical curricula at both undergraduate and clinical postgraduate levels. *Maqasid Shariah* offers a holistic view of life, focusing on maintaining good conditions and ensuring the welfare of all humankind, while preventing harm, evil, or injury. Nevertheless, there is currently no available questionnaire or assessment tool to verify the understanding of the community or physicians regarding *Maqasid Shariah* in the context of medical practice. Developing such a scale is crucial for analyzing feedback and assessing people's understanding of the concept, enabling improvements in future training or practice. Therefore, this study aims to develop and validate a questionnaire to assess knowledge of *Maqasid Shariah* in medical applications.

## Materials and methods

### *Study instrument and content validation*

The objectives of this study are to measure the reliability and construct validity of a newly developed questionnaire for the *Maqasid Shariah Knowledge Scale*. For this questionnaire, two domains were proposed and identified. The first domain consists of general questions about the respondent's background in Islamic education and exposure to *Maqasid Shariah*; the second domain

includes 14 items covering general topics related to *Maqasid Shariah*.

The items were developed through discussions and expert opinions from a panel of six members: three experts in *Maqasid Shariah*, two experts in questionnaire development and validation, and one expert in linguistics. These experts ensured the content and structural validity of the questionnaire. All panel members agreed that the items effectively covered key areas of *Maqasid Shariah* knowledge and were suitable for local contexts.

#### *Sample Size.*

The sample size is calculated based on the subject-to-variable ratio of 1:10 according to the Nunnally method [5]. As there are 20 items in this questionnaire, the required sample size is about 200 respondents.

#### *Face Validation*

Face validity of the newly developed questionnaire was assessed by conducting pre-testing among ten Malay-speaking, Muslim graduate students. The aim of this pretest is to ensure the readability and comprehensibility of the questions. The participants were required to answer the questions completely via a self-administered method initially and comment on each question if necessary. The comments were then discussed among the researchers.

#### *Questionnaire Distribution, Reliability and Validity Testing*

The final version of the questionnaire was then distributed to 254 students and staff of the medical faculty at a local university. These individuals met the inclusion criteria of the study, which are: 1) currently an active staff member or student of the medical faculty; 2) adult aged over 18 years; 3) Malaysian; 4) Muslim; and 5) able to converse in English. Verbal and written consent were obtained through their willingness to sign in for the online pre-test and submit the answers. The respondents were informed that their participation in the study was completely

voluntary and that they could withdraw their consent to participate at any time without the need for an explanation.

Demographic data of the participants were summarized using descriptive statistics with means and standard deviations for continuous variables and counts and percentages for categorical data. For reliability testing, Cronbach's alpha was selected to measure the internal consistency among the items [6]. A score above 0.7 for Cronbach's alpha was considered highly consistent, while a value of 0.40 – 0.69 was considered moderately consistent [7]. The higher the value of Cronbach's alpha, the more reliable the questionnaire items [6-8]. Factor analysis was performed to measure the questionnaire's construct validity. The best items representing each component were identified. A Kaiser-Meyer-Olkin value of more than 0.7 and a significant Bartlett's test of sphericity ( $p$  value < 0.001) are needed to provide significant components in the factor analysis [9].

#### *Ethical Approval*

The study has been approved by the IIUM Research Ethics Committee (IREC 2022-113).

## **Results**

#### *Demographic data of the respondents*

A total of 254 staff and students responded. All of them were Muslims, and 99% were Malaysian. 69.2% were female. The age of respondents ranged from 19 to 51 years (Table 1).

#### *Reliability analysis*

The Cronbach's alpha value of the 20 items was 0.647 which is acceptable. After discussion with experts and team members, items with low corrected item total correlation (below 0.19) namely items number 11,12, 14, 16, 17,18,19 and 20 were removed in view of poor discriminating value [10]. Table 2 shows the corrected item-total correction for each item before the deletion of those items mentioned.

### *Construct validity*

Factor analysis of the remaining 12 items showed an acceptable value for the Kaiser-Meyer-Olkin measure of sampling adequacy (0.761) and a significant Bartlett's Test of Sphericity ( $< 0.001$ ), which gave rise to three domains. However, by taking the eigenvalue of 2.0 on the scree plot (Figure 1), the graph levels off at component number 2. An eigenvalue of 2.0 was chosen based on Monte Carlo parallel analysis. Furthermore, by looking at the number of items without overlapping domains in factor analysis as shown in Table 3, the ideal number of retained domains is indeed two (Domain 1 and Domain 2). After discussion with the experts, Domain 3 was removed as the items in this domain were low in number and already present in Domain 1 and Domain 2. The first domain consists of six general questions pertaining to the exposure of respondents to *Maqasid Shariah*. The second domain consists of six items of general knowledge on *Maqasid Shariah* components. Reliability analysis of the remaining 12 items revealed a high Cronbach's alpha score of 0.700, with values of 0.747 for the first domain and 0.578 for the second domain.

### **Discussion**

The purpose of this study is to determine the reliability and construct validity of the newly developed *Maqasid Shariah* knowledge scale. Reliability refers to the consistency of assessment data or scores over time [10], while validity pertains to the extent to which a concept is accurately measured in a quantitative study [11]. One of the major types of validity is construct validity, which refers to how well a research instrument measures the intended construct. In this research, we aim to evaluate whether the domains included in the newly developed questionnaire effectively assess knowledge of *Maqasid Shariah* among the medical faculty community.

Regarding the demographic characteristics of the respondents, the median age was 32 years, which falls within the middle age range for Malaysians [12]. All respondents were Muslim and Malay, key communities for applying the concept of *Maqasid Shariah* in their daily activities. This target population belongs to the medical community, which often relies on Islamic teachings when making decisions regarding medical procedures and issues. As frontline healthcare providers, doctors regularly face questions about precautions and prohibitions from an Islamic perspective [13]. It is important to include both staff and students in this study to gain a more comprehensive understanding of the questionnaire items and *Maqasid Shariah* topics. Both faculty and students should share a similar level of understanding and application of *Maqasid Shariah* principles.

Although it has been suggested that good knowledge does not always translate into practical skills among respondents, many studies emphasize the need to measure the knowledge of *Maqasid Shariah* among Muslim professionals [14, 15]. This general assessment is valuable for evaluating the effectiveness of university education programs, particularly for academicians, before it can be fully appreciated by students [16]. A sufficient level of knowledge is essential for ensuring a holistic approach to patient care that aligns with Islamic teachings [17]. Integrating *Maqasid Shariah* into medical practice can enhance cultural sensitivity in healthcare, thereby improving patient trust and compliance. Furthermore, *Maqasid Shariah* can influence the creation of public health policies that are equitable, just, and aimed at enhancing the overall well-being of society. Indirectly, it also emphasizes preventive measures to protect health and prevent illness, thereby reducing the burden on healthcare systems [1-4].

The *Maqasid Shariah* Knowledge Scale uses a simple true/false answer format, making it accessible to the general public, regardless of their formal educational background. The questionnaire is also practical and easy to

complete for respondents of any background, as it does not include sub-questions or multiple statements per item, which are relatively more complex and require critical thinking [18, 19]. Each item consists of a brief statement, making it suitable for any staff to answer, whether they are specialists, consultants, or support staff. Additionally, the entire questionnaire takes only five to ten minutes to complete. This study validated the *Maqasid Shariah* scale and identified three significant domains based on exploratory factor analysis. The three domains include background-related items, general knowledge-related items, and a combination of background and knowledge-related items for the third domain. Structurally, these three domains represent common concepts and questions regarding basic *Maqasid Shariah*. The items collectively cover essential topics that healthcare providers should understand, making the scale useful for assessing the understanding of both staff and students regarding *Maqasid Shariah* principles.

Despite several domains being retainable, ranging from one to three factors based on the combination of the scree plot, Kaiser's criterion, and parallel analysis (using Monte Carlo principal component analysis), we decided to retain two significant domains after rerunning the factor analysis by manually setting the number of factors. Initially, the rotation matrix method identified three domains; however, the factor loadings (in Table 3) reveal that some items strongly represent Domain 1 (Items 1-6), others strongly represent Domain 2 (Items 7-15), while some items are fairly loaded on both Domain 2 and Domain 3. To demonstrate that only two domains adequately represent the entire questionnaire, another factor loading was conducted, forcing the grouping into two domains (Table 4). The factor loadings for each item ranged from 0.4 to 0.8, confirming that the final set of items can be categorized into only two domains [20,21]. Notably, the two domains produced acceptable Cronbach's alpha values, with no need to reduce or omit any items. If a third

domain were retained, it would consist of a mixture of items from the first and second domains. The forced number of factors set by the authors is a valid step in factor analysis, given their theoretical knowledge and pre-research overview of the appropriate number of factors to represent the items [22,23].

The final 12 items demonstrated acceptable internal consistency (Cronbach's alpha = 0.700). The first six items (first domain) represent respondents' personal backgrounds and past experiences related to the *Maqasid Shariah* principles. Meanwhile, the other six items (second domain) adequately represent the concept of the *Maqasid Shariah*, pertaining to faith, life, intellect, lineage, and property [1-3]. Assessing the underlying background of the patients is important to correlate it with their understanding of the *Maqasid Shariah* [3]. Six items per domain are indeed acceptable in comparison to other studies [24,25].

A key limitation is that the respondents are confined to the medical community, meaning this version is validated for individuals with a medical background, particularly doctors. However, since the questionnaire includes general items, it may also be applicable to other populations without modification. Additionally, the scenarios are limited to general contexts and do not cover specific medical disciplines, such as surgical, obstetric, or paediatric fields. The questionnaire currently consists of only one form of response, so future iterations could incorporate items that allow for one best-answer responses.

## Conclusion

The *Maqasid Shariah* Knowledge Scale has demonstrated validity and reliability as a quick assessment of knowledge among our local academic and medical staff. This brief and respondent-friendly questionnaire can be easily completed through a self-administered method, making it suitable for the Malaysian population in clinical practice. It serves as a tool to enhance

understanding and practical knowledge of applying the concept in daily medical tasks.

### Acknowledgement

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### Conflict of Interest

None

### Authors contribution

MSES – main idea, methodology, writings; AAH – content expert; ACA – content expert; RM – validation and analysis expert; NMY – content expert; AA - content expert; MFMI - content expert

Table 1. Demographic data of the respondents

Variables		Frequency	Percentage
Age		Median 32	(19-51)
Gender	Male	84	33.1
	Female	170	66.9
		254	
Department	Family Medicine	20	7.9
	Anaesthesiology	19	7.5
	Internal Medicine	11	4.3
	O&G	18	7.1
	Radiology	7	2.8
	Orthopedic	7	2.8
	Psychiatry	14	5.5
	Surgery	2	.8
	Others	4	1.6
Undergrads		152	59.8
Profile	Staff	40	15.7
	Undergrads	152	59.8
	Postgrads	62	24.5

Table 2. Reliability analysis of 20 items before deletion of items 11,12, 14, 16, 17,18,19 and 20

	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted	Cronbach's Alpha
1. History of attending Islamic Primary School	0.258	0.632	
2. History of attending Secondary Primary School	0.339	0.620	
3. History of attended session or lecture on Maqasid Shariah	0.466	0.603	
4. Have you previously applied the principle of Maqasid Shariah in your daily life or career?	0.394	0.616	
5. Have you read anything previously regarding Maqasid Shariah?	0.462	0.607	
6. Do you have access to any reference or reading materials regarding Maqasid Shariah?	0.368	0.616	
7. Maqasid Shariah is founded on modern principles.	0.192	0.639	0.647
8. Maqasid Shariah is confined to Muslim settings.	0.195	0.640	
9. Maqasid shariah focuses on the issues facing mankind in the hereafter.	0.214	0.638	
10. The three main rules of Shariah are Ihsan, Aqidah and Akhlak.	0.414	0.610	
11. Sanctions relating to belief (Al-Ahkam al-I'tiqadiyah) are the rules related to the pillars of faith.*	0.051	0.649	
12. Rules relating to financial transactions are under sanctions relating to morals and ethics (Al-Ahkam al-Akhlak).*	0.117	0.651	
13. Essential Maqasid (Darruriyyah) includes religion, life, environment, wealth and progeny.	0.318	0.623	
14. Protection of religion includes defending Islamic faith in our daily affairs.*	0.168	0.644	
15. Protecting everyone's life is obligatory for every Muslim first, compared to other communities.	0.240	0.634	
16. Prohibition of liquor and substance abuse is included under the protection of Maqasid.*	-0.035	0.655	

17. Safeguarding chastity is one of the elements in the protection of progeny.*	-0.056	0.652
18. Madam Salmah, a 60-year-old woman with underlying diabetes mellitus type 2, is currently fasting during the holy month of Ramadhan. Within six hours of fasting, she develops coldness, dizziness, lethargy, and extreme thirst. She noted her capillary blood sugar was 2.8 mmol/L. Which of the following is the best option for her?*	0.034	0.651
19. Madam Rosnah, a 33-year-old teacher, comes with her son, who has been diagnosed with hand-foot-and-mouth disease. Her son needs to be isolated at home. She therefore requests that you provide medical leave for her in order to take care of her son at home. She is otherwise asymptomatic without any medical illness, upon your assessment. What is the best option?*	0.178	0.655
20. Mr. Razak is a quadriplegic patient, and he wants to perform Zohor prayer. He asks a female staff nurse to assist him in taking ablution. Which of the following interventions is appropriate?*	0.059	0.648

\* Items deleted in view of low Corrected Item-Total Correlation value

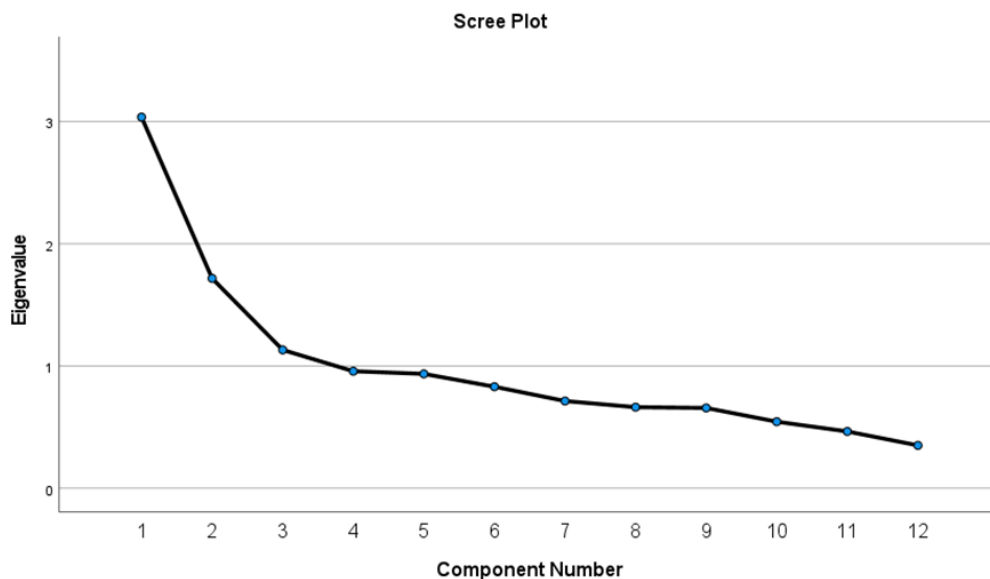


Figure 1. Current structure in managing diabetic foot.

Table 3. Factor analysis of 12 items.

	1	2	3
Item 1	0.228		0.613
Item 2	0.419		0.588
Item 3	0.776		
Item 4	0.777		
Item 5	0.793		
Item 6	0.697		
Item 7		0.360	0.386
Item 8		0.237	0.628
Item 9		0.638	
Item 10		0.765	
Item 13		0.587	0.294
Item 15		0.533	

Rotation Method: Quartimax with Kaiser Normalization.

Table 4. Rotated component matrix (factors loading forced into 2 groups)

	Domain	
	1	2
Item 1	.402	
Item 2	.581	
Item 3	.775	
Item 4	.760	
Item 5	.785	
Item 6	.651	
Item 7		.444
Item 8		.404
Item 9		.565
Item 10		.526
Item 13		.635
Item 15		.757

Extraction Method: Principal Component Analysis.; Rotation Method:

Varimax with Kaiser Normalization.;

a. Rotation converged in 3 iterations.

b. Factors loadings forced into two group



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ORIGINAL ARTICLE

## Gray Value Variation in Chest Imaging: Effects of Fabric Materials in Computed Radiography.

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### Abstract

**Introduction:** It is generally accepted that patients undergoing chest X-rays may wear their own tops if the clothing is free from metal and is plain. However, there are concerns that such clothing could introduce artifacts, potentially impacting image interpretation. Consequently, it is common practice to require patients to change into provided garments. This study aims to assess the impact of different fabric types on grey-level values in chest radiographs.

**Methods:** A phantom was positioned against an erect bucky in the posteroanterior position and exposed to X-rays under four conditions: a control setup without any fabric, and three experimental setups involving a radiography examination gown, a cotton t-shirt, and a polyester t-shirt. For each condition, exposure factors were set at 3 mAs and 105 kVp, with three repetitions per condition. The grey values in the processed radiographs were then calculated at seven anatomical points.

**Results:** The radiographic examination gown consistently showed lower mean grey values at Points 4, 5, 6, and 7, indicating a reduction in mean grey value compared to the control. . These reductions were statistically significant, with the most pronounced change observed at Point 7 (Point 4 = 0.47%,  $p = 0.010$ ; Point 5 = 1.92%,  $p = 0.017$ ; Point 6 = 0.5%,  $p = 0.021$ ; Point 7 = 4.28%,  $p = 0.018$ ).

**Conclusion:** Hospital gowns can reduce the mean grey values in certain chest areas on radiographs, potentially affecting image interpretation.

**Keywords:** *artifact, chest X-ray, fabric, gray values, hospital gown, image quality.*

## Introduction

In modern medical practice, diagnostic imaging is essential for accurately assessing and managing a wide range of health conditions. X-ray radiography is one of the most commonly used imaging techniques, providing critical insights into the internal structures of the human body. When performing chest X-ray examinations, proper patient preparation is crucial for obtaining high-quality images and ensuring accurate diagnoses. A key aspect of this preparation involves carefully considering the clothing worn by the patient during the procedure.

Several studies have investigated the impact of different fabrics on the quality and safety of X-ray imaging, focusing on various body regions. Research by Choi et al. revealed that both hospital and X-ray department gowns significantly altered the gray levels in digital X-ray images, though they did not introduce artifacts [1]. Similarly, Amran & Mohd Rais (2018) found that heavy, dull satin, high-quality lycra, and moss crepe fabrics did not cause artifacts or significantly affect the image quality of knee radiographs [2]. These findings suggest that certain fabrics may be safely worn during X-ray examinations without compromising the image quality.

However, in the context of chest X-rays, the situation becomes more complex. The low contrast resolution of chest radiographs makes them particularly susceptible to issues caused by artifacts, which can easily be mistaken for abnormalities. Even subtle alterations in gray levels caused by certain fabrics can lead to misinterpretations, potentially resulting in incorrect diagnoses or the need for repeat imaging.

Given the high stakes involved, the prevailing practice is to change the patient's clothing before a chest X-ray as a preventive measure against these risks. Despite evidence suggesting that some fabrics might not introduce artifacts, the potential for misinterpretation due to altered gray levels underscores the importance of this precaution.

Therefore, this study aims to investigate the effects of different fabric materials on gray levels in chest X-rays.

## Methods

For the control setup, an N-1 Lungman Multi-Purpose anatomical chest phantom without clothing was positioned against an erect bucky in the Posteroanterior (AP) position with a source-to-image distance (SID) of 180 cm. The X-ray beam was centered at the level of the seventh thoracic vertebrae. The phantom was exposed to X-rays at 3 mAs and various 105 kVp using a Siemens Multix Polydoros IT 55 machines. The exposure was repeated three times.

For the experimental setup, the exposure was made with the phantom wearing a radiography examination gown, a 100% cotton T-shirt, and a 100% polyester T-shirt. All fabrics were plain, free from metal, and wrinkle-free. Other settings were kept constant for both setups.

After exposure, the cassettes were processed using a Carestream DIRECTVIEW Classic CR system reader. The processed radiographs were transferred to a laptop computer for gray value analysis using ImageJ.

For the gray value analysis, seven anatomical points were selected, according to the points used for tuberculosis diagnosis by the Japan Anti-Tuberculosis Association (Figure 1).

The radiographs were zoomed to 300% to ensure accuracy in pinpointing the points. The location of each point for the first radiograph was noted for its y and x coordinates so that the points is constant throughout the study. A selection tool named Rectangular Selection Tools from the software was used to maintain the same size of gray scale evaluation on each point, minimizing the error of measuring the incorrect region of interest (Figure 2). The width and height of the selection tool were kept at 100 for all point

measurements of all radiographs to ensure data collection consistency.

The data were analysed using GraphPad Prism 10. A two-way ANOVA with multiple comparisons was used to compare the mean gray values of various fabric materials with the control group at each point. A significance level was set to  $p < 0.05$ .

## Results

A total of 84 images have been acquired in this study. The data, summarized in Table 1 reported the mean grey value when exposing the phantom with the radiography examination gown t-shirt (cotton), and t-shirt (polyester). The control provides the baseline measurements for each anatomical point, ranging from 1,334.20 at Point 1 to 3,500.86 at Point 6.

Radiographic examination gown consistently showed lower mean grey values at Points 4, 5, 6, and 7, indicating a reduction in mean grey value compared to the control. (Figure 3). These mean grey value reductions are significant with the most pronounced at point 7 (Point 4= 0.47%, p-value= 0.010; Point 5= 1.92%, p-value= 0.017; Point 6= 0.5%, p-value= 0.021 and Point 7= 4.28%, p-value=0.018).

In contrast, other garment conditions, such as T-shirt cotton and T-shirt polyester, did not yield significant differences, with the gray value differences generally closer to zero or positive at other points.

## Discussion

This study aims to assess the impact of examination gowns on the quality of radiographic images by comparing the gray values of phantom images obtained with and without various fabrics (radiography examination gown, cotton and polyester t-shirt). In radiological imaging, the "gray value" denotes pixel intensity, which is

directly correlated with tissue density as determined by X-ray attenuation [3]. Higher gray values indicate denser tissues such as bone, while lower values correspond to less dense tissues like air or fat.

The generation of gray values in radiological images is influenced by several factors, including X-ray type and energy, tissue characteristics, and imaging device settings such as tube voltage and exposure time [3,4]. The presence of fabric or other materials between the imaging receptor and the tissue being scanned introduces an additional layer of attenuation, affecting the recorded mean gray value [5]. As X-rays traverse the fabric, their energy is partially absorbed or scattered, resulting in lower-energy X-ray photons reaching the detector. The extent of this attenuation is contingent upon the fabric's material properties, including thickness, density, and composition [6]. Fabrics with higher density, such as heavy cotton or synthetic materials like polyester, exhibit a greater capacity for X-ray absorption.

This study found that hospital gowns significantly reduce mean gray values, whereas other fabrics did not yield a comparable effect. This suggests that hospital gowns possess a higher attenuation coefficient relative to T-shirts made of cotton or polyester. Hospital gowns, typically designed for durability and patient protection, are often constructed from thicker, denser fabrics or multiple layers [7], while T-shirts are generally made from lighter, single-layered fabrics with a looser weave. A similar finding was reported by Ji hoon et al. (2018), who examined the impact of hospital gowns on chest radiographs and concluded that dense fabric gowns may induce minor but measurable changes in image contrast, though these changes were not usually significant enough to compromise diagnostic outcomes. [8] Kusuktham et al. (2016) emphasized that the material composition and thickness are critical factors influencing the extent of X-ray absorption [9].

Radiography examination gowns can cause a reduction in mean gray values, which may have practical implications for diagnostic decisions, particularly in radiographic imaging where contrast and detail are critical. While this reduction may not be perceptible to the human eye if the optical density (OD) changes are below the visual detection threshold, it can still impact the ability to distinguish between different tissue densities or detect subtle abnormalities [10]. Regions corresponding to points 4, 5, 6, and 7 on the chest encompass critical organs, where common pathologies like pulmonary tuberculosis, pleural effusion, and other lung-related abnormalities are often found. Certain pathologies can increase lung tissue density. For example, tuberculosis lesions typically increase tissue density due to granuloma formation and fibrosis, resulting in higher gray values on a radiograph [11,12]. If a thicker radiography examination gown is worn during imaging, the gown's attenuation could lower the mean gray value, potentially obscuring subtle changes in tissue density [13,14]. This is particularly concerning in the early stages of the disease, especially in areas where high contrast and sensitivity are required.

## **Conclusion**

In conclusion, the finding that radiography examination gowns can reduce mean gray values

is significant. It indicates that the use of such gowns in radiographic imaging may potentially interfere with the detection and accurate diagnosis of pathologies at critical anatomical points. Therefore, careful consideration of gown material and thickness is essential in clinical settings to ensure that diagnostic accuracy is maintained, particularly when dealing with conditions that alter tissue density.

## **Conflicts of interest**

The authors have no conflict of interest to declare.

## **Acknowledgement**

The authors would like to thank science officers, laboratory technicians for providing the equipment used for this study.

## **Authors Contribution**

NSA and NFI conceptualized and designed the study. NSA collected the data. NFI analysed the data and prepared the manuscript.

## **Ethical statement**

The study does not involve human subjects or their identifiable data. Therefore, it is exempt from the requirement for ethical approval.



Figure 1. The anatomical points used for gray scale evaluation based on Japan Anti-Tuberculosis Association Seven points (Choi et al., 2016). Point 1: The high density area between rib 5 and rib 6 on the right lung; Point 2: The overlapped area of anterior rib 5 and posterior rib 6 on the right of the periphery of lung; Point 3: The transmission part of mediastina carina; Point 4: the area right below mediastina carina; Point 5: The abdominal aortic right at the descending aorta; Point 6: Thoracic vertebrae part 10 and 11 of the shade area of heart; Point 7: The abdominal aortic at the center of right diaphragm.

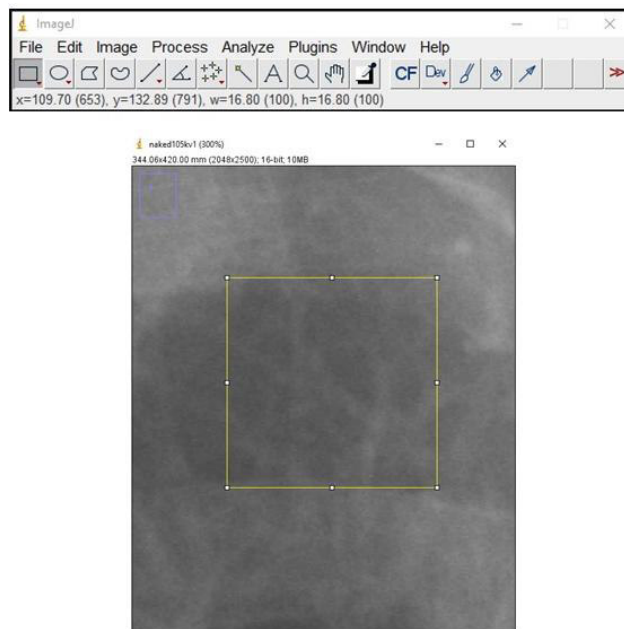


Figure 2. Radiograph was expanded 300% and Rectangular Selection Tool was selected to measure the gray value of each point using ImageJ.



Table 1. Mean gray values at seven points in the chest X-ray after irradiation without fabric (control) and with fabric (radiography examination gown, cotton, and polyester t-shirt).

Anatomical point	Control		Radiography examination gown		T-shirt cotton		T-shirt polyester	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Point 1	1334.20	4.36	1345.17	0.21	1327.07	1.24	1326.74	3.28
Point 2	2237.61	24.08	2286.76	9.98	2236.44	18.68	2242.50	2.47
Point 3	3085.62	4.46	3084.90	1.48	3082.03	2.57	3078.47	1.68
Point 4	3363.45	3.54	* 3347.63	2.75	3363.29	1.06	3360.78	0.70
Point 5	3035.96	14.39	* 2977.61	14.24	3019.12	9.76	3008.66	6.07
Point 6	3500.86	4.88	* 3483.02	4.18	3502.79	1.37	3502.71	1.08
Point 7	2824.25	31.06	* 2703.21	17.85	2827.51	9.33	2825.16	4.90

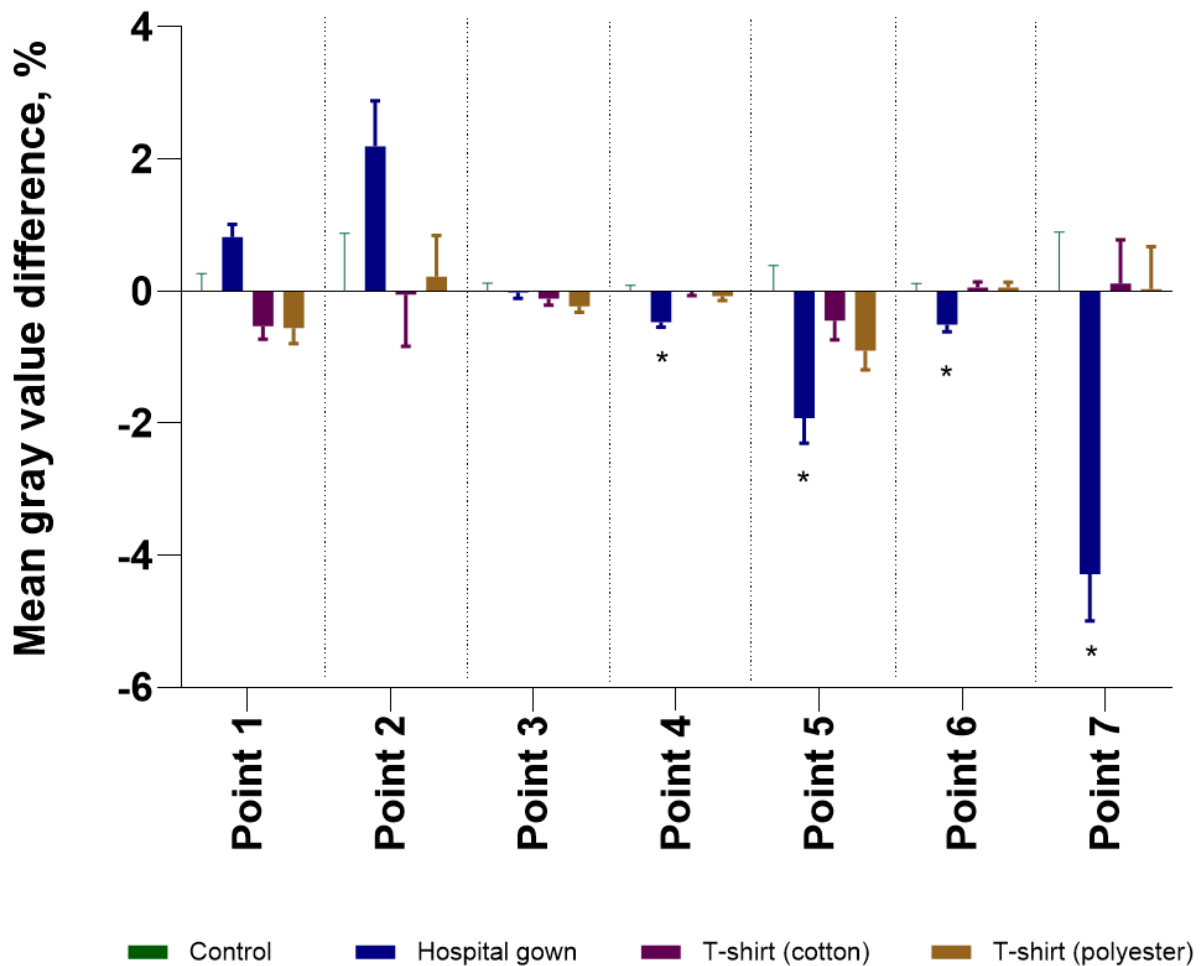


Figure 3. Percentage differences between the mean gray values for the phantom without fabric (control) and with fabric (radiography examination gown, cotton, and polyester t-shirt) at seven points in the chest X-ray.

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ORIGINAL ARTICLE

## Study of E-Cigarette Usage among University Students in Perak using the Modified E-Cigarette Evaluation Questionnaire (MECEQ).

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### Abstract

**Background:** E-cigarettes are devices of growing popularity worldwide, primarily containing nicotine. Despite the increasing number of e-cigarette users in Malaysia, there remains insufficient data on the subjective effects of e-cigarette use among young adults. This study aims to examine these effects among university students in selected universities in Perak, utilising the Modified E-Cigarette Evaluation Questionnaire (MECEQ). **Methods:** A study sample size of 236 respondents was determined using the OpenEpi online software, targeting e-cigarette users from six universities in Perak. The questionnaire, distributed via Google forms, and Whatsapp, comprised three sections: sociodemographic information, smoking status, and the Modified E-Cigarettes Evaluation Questionnaire (MECEQ). Data analysis was conducted using IBM SPSS version 23, employing frequency and chi-square tests to identify associations between e-cigarette use and various factors. A  $p$ -value of  $<0.05$  was considered statistically significant. **Results:** The study revealed that most university students in Perak who participated were male ( $n=218, 92.4\%$ ), with a household income below RM 2000 ( $n=196, 83.1\%$ ). Most participants had been using e-cigarettes for 1-5 years. Analysis of the MECEQ showed a moderate average score for Vaping Satisfaction, while Psychological Reward, Enjoyment of Respiratory Tract Sensation, and Aversion had low average scores. **Conclusion:** This study highlights that e-cigarette users aged 18-24 scored moderately on Vaping Satisfaction. This suggests that colleges and universities can promote e-cigarette cessation through peer-led support, health campaigns, and incorporating the topic into curricula. Incentives and tobacco-free programs could also encourage quitting. Given the fairly mild subjective effect of e-cigarette use, authorities should prioritise on educating students about the harmful ingredients found in e-cigarettes. More research is needed to determine the health concerns in this population.

**Keywords:** E-cigarette, Modified E-Cigarettes Evaluation Questionnaire (MECEQ), Subjective effects.

## Introduction

E-cigarettes are devices that allow users to inhale an aerosol containing nicotine, flavourings, and other additives. Despite differing in design, they function similarly and share common components [1]. The Centers for Disease Control and Prevention (CDC) states that e-cigarettes are known by various terms, such as "electronic nicotine delivery systems (ENDS)," "vape pens," and "e-cigs." Some resemble traditional cigarettes, cigars, or pipes, while others mimic everyday items like pens and USB drives [2]. According to the World Health Organization (WHO), e-cigarettes are detrimental to health and lack safety assurances. Although the long-term effects are not yet fully understood, they are known to emit toxic and carcinogenic substances, elevating the risk of heart and lung diseases [3].

In 2020, a study revealed 5.4% of Malaysian adults were daily e-cigarette users [4]. Among this group, more than half had nicotine in their e-cigarettes, leading to reports of strong nicotine addiction. The importance of ongoing surveillance is emphasised to track potential changes in e-cigarette initiation rates among youth, young adults, and non-smokers in Malaysia. Monitoring these trends is crucial for understanding the evolving landscape of e-cigarette use in the country [4].

In 2018, a study among university students in Malaysia uncovered widespread e-cigarette use. Participants were categorized into three groups: exclusive e-cigarette users, dual users of cigarettes and e-cigarettes, and non-users. Adverse effects such as dizziness, cough, and headaches have been reported. The study also revealed varied motivations for e-cigarette use, with 57.8% viewing them as a tool for smoking cessation, while others saw them as a means to enhance self-image or partake in social activities [5].

In 2021, research indicated that 69.08% of respondents were aware of the negative health impacts of using e-cigarettes use [6]. Additionally,

43.32% of participants believed that e-cigarette use could lead to significant health issues for smokers. Overall, the study aimed to discourage the use of e-cigarettes by enhancing university students' awareness and knowledge [6].

Consumer preferences for the choice of flavoured e-cigarettes varied among consumers demonstrating variations across age groups and smoking status, with flavoured options being preferred. The research indicated that certain flavours were linked to a reduced perception of harm, while tobacco flavour was associated with increased perception. Furthermore, specific flavour chemicals and sweeteners employed in e-cigarettes raised potential toxicological concerns. Lastly, preferences for nicotine strength and types were influenced by factors such as smoking status and e-cigarette use history. These findings highlighted the diverse factors influencing consumer choices and perceptions in the realm of e-cigarettes [7].

The government, through its National Strategic Plan for the Control of Tobacco and Smoking Products 2021-2030, has outlined various mechanisms to combat the use of smoking products, including e-cigarettes. However, at the moment there is inadequate data on the usage and practice of e-cigarettes among youth in Malaysia which is crucial for improving strategies to curb this habit.

Thus, this study aimed to analyse the subjective effects of e-cigarettes usage among university students in Perak and its associated factors using MECEQ (Modified E-Cigarette Evaluation Questionnaire) [8].

## Materials and methods

This was a cross-sectional study on the effects of E-cigarettes among 236 university students from selected universities in Perak, including Universiti Teknologi Petronas, UITM Seri Iskandar, Universiti Pendidikan Sultan Idris, Universiti Kuala Lumpur Royal College of Medicine Perak (UniKL RCMP), Universiti

Kuala Lumpur Malaysian Institute of Marine Engineering Technology (UniKL MIMET) and Institut Pendidikan Guru Malaysia Kampus Ipoh. The sample size was calculated using the software Openepi.com. With a population size of 25,000, we initially received 378 responses. A post-hoc analysis was conducted, filtering out respondents who did not smoke e-cigarettes, which resulted in 236 responses. Recalculation, utilising an anticipated frequency of 5.4% for the prevalence of E-cigarette smokers showed a 99.9% confidence level with a sample size of 236 [4]. The study employed a non-probability, purposive sampling method. The inclusion criteria required respondents to be e-cigarette smokers, aged 18 to 24 and enrolled in selected universities. Exclusion criteria applied to university personnel who were not students. All data were collected via an online questionnaire (Google Form) distributed through WhatsApp. The questionnaire consisted of three sections: Section A, Section B, and Section C. Section A covered the socio-demographic details of the respondents. Section B addressed the status of smoking, and Section C contained the validated Modified E-cigarette Evaluation Questionnaire (MECEQ) from a study by Morean et al, which consists of four subscales: Vaping Satisfaction (3 items), Psychological Reward (6 items), Enjoyment of Respiratory Tract Sensation (1 item) and Aversion (2 items) [8]. Every item was scored on a 7-point Likert scale ranging from 1 (Not at all) to 7 (Extremely). The questionnaire was previously validated by Morean et al., and Cronbach's alpha for each item ranged from 0.71 (SE 0.02) to 1.00 (SE 0.00 [8]. For this study, the questionnaire was pre-tested by 10 university students before the actual data collection to ensure the questionnaire was clear, appropriate, and no redundancy. These 10 students were not included in the main study analysis. All data collected from the questionnaire were processed using IBM SPSS version 23 for analysis. The Chi-Square test was used to identify the association of categorical data. P value < 0.05 was considered statistically significant. ANOVA test was used to analyse the differences between two or more

independent variables on a continuous or ordinal dependent variable.

## Results

### Section A: Socio-demography

After data cleaning, 236 respondents were selected from 378 responses received. In Table 1, the majority were found to be under 21 years old (n=119, 50.4%) with most respondents identified as male (n=218, 92.4%) and female (n=18, 7.6%). The predominant race was Malay (n=213, 90.3%) and non-Malay (n=23, 9.7%). Most respondents were from public universities (n=145, 62.7%), with the remaining enrolled in private institutions (n=89, 37.3%). In terms of household monthly income, the majority of respondents came from families that earned less than RM2000.00 (n=196, 83.1%), while the remaining earned more (n=40, 16.9%).

### Section B: Smoking status

The smoking status of the 236 respondents is outlined in Table 2. It was found that 157 respondents (66.5%) had used e-cigarettes in the past month while the remaining 79 respondents (33.5%) had not. Among e-cigarette users, 182 respondents (77.1%) never smoked regular cigarettes before, in contrast with 54 respondents (22.9%) who had used usual cigarettes. Only 43 respondents (18.2%) intended to quit smoking by using e-cigarettes. Fifty-one (21.6%) respondents were dual users, while 185 (78.4%) exclusively used e-cigarettes. Over half (66.5%) had been using e-cigarettes for 1 to 5 years. Friends' influence (35.2%), stress relief (27.0%), and saving money (18.4%) were identified as the primary reasons for e-cigarette use. The majority used "pod vapes or pod mods" (69.9%), followed by "vape pens or tank system" (19.7%).

## Section C: MECEQ Scores

Table 3 displays the frequency and percentage of MECEQ items among respondents, while Table 4 outlines the items by subscale. The highest mean scores were noted for "E-cigarettes or vapes are satisfying" (4.29) in the Vaping Satisfaction category, "Did you enjoy smoking e-cigarettes?" (3.69) in Psychological Reward, "Vaping makes me dizzy" (2.05) in Aversion, and "I enjoy the sensations of vaping in my throat and chest" (2.89) in Enjoyment of Respiratory Tract Sensation. A summary of the MECEQ questionnaire mean scores can be found in Table 5: Vaping Satisfaction (4.29), Psychological Reward (2.70), Enjoyment of Respiratory Tract Sensation (2.89), and Aversion (1.96). The scores were categorized into high, moderate, and low levels according to Bloom's cut-off points: high ( $\geq 80\%$  of the maximum score,  $\geq 5.6$ ), moderate (60-70% of the maximum score, 4.2-5.6), and low (below 60% of the maximum score,  $< 4.2$ ). Therefore, vaping satisfaction is considered moderate, while psychological reward, enjoyment of respiratory tract sensations, and aversion are rated as low.

As shown in Table 6, significant associations were found between e-cigarette use for smoking cessation and both the university of study ( $p = 0.037$ ) and household income ( $p < 0.001$ ). Students from public universities and from low household income ( $< \text{RM } 2000$ ) were associated to use e-cigarettes without intention to stop smoking. Table 7 indicated that no significant associations were found between e-cigarette usage and sociodemographic characteristics, except for gender ( $p = 0.010$ ). Table 8 displays the associations between the duration of e-cigarette use and sociodemographic characteristics. Significant associations were observed for all variables except the university of study. Factors such as being above 21 years old ( $p < 0.001$ ), male gender ( $p < 0.001$ ), Malay race ( $p = 0.039$ ), and a household income of below RM2000 ( $p < 0.001$ ), were associated with e-cigarettes use for 1-5 years.

## Discussion

### Association Between Sociodemographic Factors and E-Cigarette Usage

It is difficult to compare with the four previous studies on e-cigarettes usage in Malaysia [4,5,9] with this study, as those studies had larger sample sizes, except for Zainal Abidin N et al [9], and included older age groups. Therefore, this study is limited to university students aged 18-24, and its generalisability is confined to this group.

A key similarity across the studies is the predominance of male e-cigarette users. In our study, males ( $n=218$ , 92.4%) significantly outnumbered females ( $n=18$ , 7.6%). This aligns with findings from Driezen P et al. [4], who reported a male majority ( $n=1024$ , 50.3%) compared to females ( $n=229$ , 16.6%), and Sharifa Ezat Wan Puteh et al. [5], where males constituted an overwhelming majority ( $n=1234$ , 94.8%) versus females ( $n=68$ , 5.2%). The strong male predominance across different studies highlights a consistent gender pattern in e-cigarette usage.

Another similarity observed was the predominance of Malay users in our study. Among our respondents, 90.3% were Malay ( $n=213$ ), compared to 9.7% non-Malay ( $n=23$ ). This is in concordance with Driezen P et al. [4], who reported that 35.5% of their e-cigarette users were Malay ( $n=619$ ), although with a larger representation of Chinese ( $n=461$ , 21.2%) and other ethnic groups ( $n=173$ , 50.0%). Our findings also mirror those of Sharifa Ezat Wan Puteh et al. [5], where the distribution of Malay users ( $n=979$ , 75.2%) far exceeded that of Chinese ( $n=98$ , 7.5%), Indian ( $n=162$ , 12.4%), and other ethnic groups ( $n=63$ , 4.8%).

Zainal Abidin N et al. [9] found that 74.8% of their respondents exclusively used e-cigarettes, which is similar to this study, where 78.4% ( $n=185$ ) were exclusive e-cigarettes users. It is assumed that the lower price of e-cigarettes compared to conventional cigarettes has influenced the young population to adopt e-cigarettes use. [9]

This study also observed that 83.1% (n=196) of respondents with household income less than RM2000 use e-cigarettes in contrast with 16.9% (n=40) of respondents with more than RM2000. This may suggest that the student's main source of financial support, such as scholarships, could play a role in e-cigarette use. More students from public universities (n=148, 62.7%) use e-cigarettes compared to those from private universities (n=88, 37.3%). Among private university students, 25.0% used e-cigarettes to quit smoking, compared to 14.2% of public university students, indicating a higher tendency in private universities. Additionally, 42.5% of respondents with household incomes over RM 2000 used e-cigarettes to quit, while only 13.3% of those with incomes below RM 2000 did. This finding suggests that higher-income individuals are more likely to use e-cigarettes as a mean to stop smoking than those with lower incomes.

#### **Association of Duration of E-Cigarette Usage with Sociodemographic Factors**

We have found significant associations between the duration of e-cigarette usage and each of the four sociodemographic characteristics (age, gender, race, and household income) (p-value < 0.05)

With regards to age, most respondents of both groups (those under and over 21 years of age respectively) reported using e-cigarettes for 1 to 5 years. However, a notable number under 21 have used e-cigarettes for over 5 years, suggesting early initiation during teenage years.

When comparing the two genders, female respondents generally started using e-cigarettes more recently (less than 1 year) compared to males, who have used e-cigarettes for 1 to 5 years. However, among younger respondents, females are more likely to have used e-cigarettes for over 5 years compared to males, implying that females feature more predominantly in those who started smoking before 21 years of age compared to their male counterparts.

In terms of the difference seen between the different races, the majority of both Malay and

non-Malay individuals were reported to have used e-cigarettes for a duration ranging from 1 to 5 years. A higher percentage of non-Malay respondents (8.7%) reported using e-cigarettes for more than 5 years compared to Malay respondents (1.4%). One more thing of note is that respondents with incomes above RM 2000 exhibited a longer duration of e-cigarette use compared to those with lower incomes.

This study highlights variances in e-cigarette usage patterns based on socio-demographic factors and provides insights into the broader implications of e-cigarette use among different demographic groups.

#### **MECEQ Scores**

Our analysis showed that respondents had moderate satisfaction with vaping. When asked about enjoyment and taste, participants expressed satisfaction, which aligns with Dawkins et al.'s (2013) online survey that described e-cigarette use as "satisfying"[10]. This highlights the positive experiences reported by university students in Perak using e-cigarettes. However, our study found that respondents reported lower psychological benefit, such as "calming," "increased alertness," "enhanced concentration," and "reduced irritability." This contrasts with previous research by Bullen et al., 2010;[11] Dawkins et al., 2013;[10] and Vansickel & Eissenberg, 2012,[12] which reported higher psychological benefits from e-cigarettes. Additionally, participants in our study reported low satisfaction with throat and chest sensations from vaping, unlike previous research by DiPiazza et al., 2020[13]. This difference may be due to social desirability bias, where participants underreported positive effects because of societal views on e-cigarettes. The lack of a Malay translation, the respondents' primary language, may also have influenced their responses. We also looked at aversion to vaping, such as nausea and dizziness. The data showed low aversion, which aligns with DiPiazza et al.'s (2020) findings, where 90% of participants reported no nausea or dizziness[13]. Similarly, Polosa et al.



(2011) found that 96.3% of respondents did not experience these symptoms after 12 weeks of vaping[14]. Our study used the MECEQ questionnaire with a 7-point Likert scale, unlike earlier studies that used a 5-point scale. Research by Finstad (2010) suggested that the 5-point scale could lead to less accurate responses, as participants might try to choose between two values[15]. The 7-point scale is seen as more precise, especially for online, unsupervised surveys like the MECEQ, potentially capturing more accurate responses from our participants. E-cigarette user perceptions taken from Surajudeen et al.,[16] showed that participants in the study used e-cigarettes to reduce the urge and act of smoking, with some reports suggesting that e-cigarettes help with the complete cessation of smoking altogether. These findings are in line with our study as efforts in the cessation of smoking were found to be one of the leading causes of e-cigarette usage.

## **Conclusion**

Most e-cigarette users in this study had been using for 1 to 5 years and were predominantly over 21 years old, male, Malay, and from low-income households. They reported moderate satisfaction with e-cigarettes and low psychological rewards, suggesting that an intervention program aimed at helping them quit could be effective. However, further research utilizing MECEQ is needed before these findings can be generalized. This study focused on 18-24-year-olds who use e-cigarettes, either exclusively or hybrid usage, and suggests universities can promote cessation

through peer-led support, health campaigns, and curriculum integration. Incentives and tobacco-free policies could further encourage quitting. With only moderate subjective effects to e-cigarette use, authorities should prioritize educating students on the harmful substances in e-cigarettes. More research is needed on the health risks in this group.

## **Conflict of interest:**

The authors declare that no conflict of interest may arise from the research publication.

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## **Ethics**

Ethical approval was granted by the UniKL Medical Research Ethics Committee, under reference number UniKLRCMP/MREC/2023-2024/SSM-010.

## **Authors' contribution:**

NHH.: Methodology, writing - Original Draft; NSA & SNHMG: Formal analysis, Writing, and Review; NAZ.: Writing, Review & Editing, Project administration. MFF: Review and Project administration; MZZ: Writing and Review; RMN: Concept, Review, Edit, and final formatting.

Table 1. Sociodemographic characteristics

		n (%)
Age	Under 21	119 (50.4)
	Above 21	117 (49.6)
Gender	Male	218 (92.4)
	Female	18 (7.6)
Race	Malay	213 (90.3)
	Non-Malay	23 (9.7)
University of study	Public	148 (62.7)
	Private	88 (37.3)
Household income	Below RM2000.00	196 (83.1)
	Above RM2000.00	40 (16.9)

Table 2. Status of smoking

		n (%)
Have used e-cigarettes recently (< 1 month)	Yes	157 (66.5)
	No	79 (33.5)
Have smoked regular cigarettes prior to smoking e-cigarettes	Yes	54 (22.9)
	No	182 (77.1)
Using e-cigarettes to stop smoking	Yes	43 (18.2)
	No	193 (81.8)
Dual user	Yes	51 (21.6)
	No	185 (78.4)
Duration of usage of e-cigarettes	< 1 year	74 (31.4)
	1 year - 5 years	157 (66.5)
	> 5 years	5 (2.1)
*Reason of starting e-cigarettes smoking	To stop smoking regular cigarettes	45 (10.0)
	To save money	83 (18.4)
	To relieve stress or anxiety	122 (27.0)
	Friends' influence	159 (35.2)
	Social media influence	43 (9.5)
*Types of e-cigarette	Cigalikes	16 (5.2)
	Pod vapes or pod mods	216 (69.9)
	Vape pens or tank system	61 (19.7)
	Box kits or box mods	14 (4.5)
	DotMod or VapeTape	1 (0.3)
	What my friends have	1 (0.3)

\*These questions are multiple response question

Table 3. Responses to MECEQ

No.	Responses	Not at all, n (%)	Very little, n (%)	A little, N (%)	Moderately, n (%)	A lot, n (%)	Quite a lot n (%)	Extremely n (%)
1	E-cigarettes/vapes are satisfying.	8 (3.4)	16 (6.8)	30 (12.7)	82 (34.7)	60 (25.4)	25 (10.6)	15 (6.4)
2	Vaping tastes good.	2 (0.8)	15 (6.4)	30 (12.7)	64 (27.1)	67 (28.4)	30 (12.7)	28 (11.9)
3	I enjoy the sensations of vaping in my throat and chest.	40 (16.9)	66 (28.0)	57 (24.2)	44 (18.6)	18 (7.6)	4(1.7)	7(3.0)
4	Did you enjoy smoking e-cigarettes?	7 (3.0)	30 (12.7)	65 (27.5)	86 (36.4)	31 (13.1)	8 (3.4)	9 (3.8)
5	Vaping calms me down.	21 (8.9)	61 (25.8)	83 (35.2)	53 (22.5)	12 (5.1)	2 (0.8)	4 (1.7)
6	Vaping makes me feel more awake.	28 (11.9)	89 (37.7)	64 (27.1)	39 (16.5)	3 (1.3)	8 (3.4)	5 (2.1)
7	Vaping makes me feel less irritable.	76 (32.2)	90 (38.1)	36 (15.3)	17 (7.2)	9 (3.8)	3 (1.3)	5 (2.1)
8	Vaping helps me concentrate.	45 (19.1)	76 (32.2)	72 (30.5)	28 (11.9)	5 (2.1)	6 (2.5)	4 (1.7)
9	Vaping reduces my hunger for food.	107 (45.3)	78 (33.1)	30 (12.7)	12 (5.1)	3 (1.3)	2 (0.8)	4 (1.7)
10	Vaping makes me dizzy.	88 (37.3)	96 (40.7)	28 (11.9)	11 (4.7)	5 (2.1)	5 (2.1)	3 (1.3)
11	Vaping makes me nauseous.	109 (46.2)	87 (36.9)	22 (9.3)	8 (3.4)	4 (1.7)	2 (0.8)	4 (1.7)
12	I enjoy vaping.	7 (3.0)	16 (6.8)	42 (17.8)	112 (47.5)	37 (15.7)	13 (5.5)	9 (3.8)

Table 4. Analysis of MECEQ by subscale

Analysis of items by subscale		Mean score (±SD)
Vaping Satisfaction	1. E-cigarettes or vapes are satisfying.	4.29 (1.37)
	2. Vaping tastes good.	4.61 (1.40)
	12. I enjoy vaping.	3.98 (1.20)
Psychological Reward	4. Did you enjoy smoking e-cigarettes?	3.69 (1.26)
	5. Vaping calms me down.	2.98 (1.18)
	6. Vaping makes me feel more awake.	2.76 (1.29)
	7. Vaping makes me feel less irritable.	2.25 (1.34)
	8. Vaping helps me concentrate.	2.60 (1.28)
Enjoyment of Respiratory Tract Sensation	9. Vaping reduces my hunger for food.	1.93 (1.21)
	3. I enjoy the sensations of vaping in my throat and chest	2.89 (1.44)
	10. Vaping makes me dizzy	2.05 (1.24)
Aversion	11. Vaping makes me nauseous	1.87 (1.18)

Table 5. Analysis of MECEQ in total

Analysis of MECEQ in total	Mean score (±SD)	Categorized based on Bloom's cut-off points*
Vaping Satisfaction	4.29 (1.32)	Moderate
Psychological Reward	2.70 (1.26)	Low
Enjoyment of Respiratory Tract Sensation	2.89 (1.44)	Low
Aversion	1.96 (1.21)	Low

\*Bloom's cut-off points: high ( $\geq 80\%$  of the maximum score,  $\geq 5.6$ ), moderate (60-70% of the maximum score, 4.2-5.6), and low (below 60% of the maximum score,  $< 4.2$ ).

Table 6. Association between e-cigarette usage and cessation of smoking

Variable		Using e-cigarettes to stop smoking		p value
		Yes n (%)	No n (%)	
Age	Under 21	22 (18.5)	97 (81.5)	0.915
	Above 21	21 (17.9)	96(82.1)	
Gender	Male	38 (17.4)	180 (82.6)	0.274
	Female	5 (27.8)	13 (72.2)	
Race	Malay	40 (18.8)	173 (81.2)	0.498
	Non-Malay	3 (13.0)	20 (87.0)	
University of study	Public	21 (14.2)	127 (85.8)	0.037
	Private	22 (25.0)	66 (75.0)	
Household income	Below RM2000.00	26 (13.3)	170 (86.7)	<0.001
	Above RM2000.00	17 (42.5)	23 (57.5)	

Table 7. Association between e-cigarette usage and sociodemographic characteristics

Sociodemographic characteristics		Have used e-cigarettes recently (< 1 month)		p value
		Yes n (%)	No n (%)	
Age	Under 21	73 (61.3)	46 (38.7)	0.089
	Above 21	84 (71.8)	33 (28.2)	
Gender	Male	150 (68.8)	68 (31.2)	0.010
	Female	7 (38.9)	11 (61.1)	
Race	Malay	144 (67.6)	69 (32.4)	0.285
	Non-Malay	13 (56.5)	10 (43.5)	
University of study	Public	105 (70.9)	43 (29.1)	0.062
	Private	52 (59.1)	36 (40.9)	
Household income	Below RM2000.00	127 (64.8)	69 (35.2)	0.213
	Above RM2000.00	30 (75.0)	70 (25.0)	

Table 8. Association between duration e-cigarette usage and sociodemographic characteristics

Sociodemographic characteristics		Duration of e-cigarettes usage			p value
		< 1 year n (%)	1 year - 5 years n (%)	> 5 years n (%)	
Age	Under 21	54 (45.4)	61 (51.3)	4 (3.4)	<0.001
	Above 21	20 (17.1)	96 (82.1)	1 (0.9)	
Gender	Male	60 (27.5)	154 (70.6)	4 (1.8)	<0.001
	Female	14 (77.8)	3 (16.7)	1 (5.6)	
Race	Malay	65 (30.5)	145 (68.1)	3 (1.4)	0.039
	Non-Malay	9 (39.1)	12 (52.2)	2 (8.7)	
University of study	Public	41 (27.7)	103 (69.6)	4 (2.7)	0.236
	Private	33 (37.5)	54 (61.4)	1 (1.1)	
Household income	Below RM2000.00	61 (31.1)	134 (68.4)	1 (0.5)	<0.001
	Above RM2000.00	13 (32.5)	23 (57.5)	4 (10.0)	



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ORIGINAL ARTICLE

## Formulation of Bergamot Essential Oil-Loaded Emulsion for Hyperhidrosis and Bromodosis: Stability and Antibacterial Assay

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### Abstract

The current marketed product of antiperspirant could temporarily block the production of excessive sweat in hyperhidrosis patients. The sweat leads to the growth of bacteria, especially in the foot area. Therefore, the chemical compound in bergamot essential oil which is linalool, able to provide antibacterial properties to the emulsion. In this study, the formulation of antiperspirant lotion containing bergamot essential oil as an antibacterial agent has been formulated and optimized at different content of oil, surfactant and aluminium chloride. The formulated emulsion has then been evaluated based on its stability and antibacterial activity. The formulation consisting of 25% aluminium chloride, 3% bergamot essential oil, 6% Tween 80, and 0.5% Phenonip (F1) was selected as the most optimum formulation. It demonstrated good stability in the overtime stability analysis, with no phase separation, no precipitation, no colour changes, and only an insignificant decrease in pH at various storage temperatures (4, 25 and 34°C). In contrast, the other formulated emulsions (F2 to F8), were found to be unstable at high-temperature storage (34°C). The stability of the emulsions was further confirmed by the positive results obtained from the accelerated and freeze-thaw stability analyses. Disk diffusion analysis indicated that the formulated emulsion with a high content of bergamot essential oil highly inhibits the growth of Gram-positive bacteria. However, the use of bergamot essential alone failed to inhibit the growth of Gram-negative bacteria due to the presence of lipopolysaccharide in the bacteria cell wall, which blocks the permeation of antibacterial agents. The newly optimized antiperspirant emulsion could overcome the problem of excessive production of sweat as well as inhibit the growth of bacteria that cause foot odour.

**Keywords:** *antiperspirant, bergamot essential oil, emulsion, formulation, topical.*

## Introduction

The skin is the largest human organ, composed of three layers, the epidermis, dermis, and hypodermis (or subcutaneous layer) which serves different functions and has distinct structures [1]. The skin's surface is a waterproof, semi-permeable membrane that acts as a skin barrier from foreign substances and pathogens. Moreover, it protects the internal organ from trauma such as chemical, thermal, and ultraviolet radiation. Skin is a natural sensor detecting the surroundings through a host of nerve endings, stabilizing body temperature, increasing metabolic function, and synthesizing vitamin D [2]. The dermis has multiple layers of connective cells located between the epidermis and the subcutaneous layer. The layer is composed of elastic tissue, collagen, vasculature, nerves, hair follicles, and sweat glands. It aids the thermoregulation of the body by secreting sweat and assists in external sensation due to the presence of the nerves ending [3].

A chronic sweating disorder called hyperhidrosis is a clinical condition where the patient secretes an excessive volume of sweat [4]. It is a dysfunction of the sympathetic and parasympathetic nervous system that seriously affects the patients' social life. Most of them experience shaking hands and unwanted sweat patches. General hyperhidrosis affects the whole body and is caused by infections, endocrine disturbances, neurological disorders, medications, intoxication, or withdrawal of alcohol or other substances. Whereas focal hyperhidrosis is the excessive production of sweat in specific areas, such as the feet, hands, armpits, and face. Primary focal hyperhidrosis can develop in healthy persons since it was suggested to have a genetic predisposition. Between 30% and 65% of patients have a positive family history. Secondary focal hyperhidrosis, on the other hand, is caused by defects in the central or peripheral nervous system [5]. The presence of carbonic anhydrase II (CAII) in the pyramidal-shaped secretory coil clear cells of the glands act as hyperhidrosis glands. It is considered a specific marker for the

sweating disorder, as this enzyme is absent in apocrine glands [4]. Topical therapy is considered the first-line treatment for focal hyperhidrosis. It contains aluminium chloride as a therapeutic agent, which block the sweat output [6]. The salt precipitates with mucopolysaccharides, resulting in damage to the luminal epithelial cells. Hence, the precipitation forms a plug that blocks the eccrine duct from secreting sweat onto the skin surface. Long-term blockage may lead to the functional degeneration of the eccrine glands with loss of secretory function [7].

Bromodosis or commonly known as foot odour is a disturbing and embarrassing condition caused by odour-forming bacteria [8]. The smell is produced by the bacteria from the breakdown of carbohydrates, peptides and fatty acids into smaller molecules. The sweat secreted by the eccrine gland does not produce odour, but the moisture environment favours the growth of the bacteria that produce odorous metabolites. In addition, the presence of exo-enzymes (lipase, protease) has a connection to the foot odour problem. Feet with strong odours have significantly more population of bacteria produced than feet with weaker odours [9]. Essential oils are an alternative and affordable antibacterial treatment for bromodosis [8]. Bergamot essential oil is widely used as antibacterial agent in pharmaceutical products [10]. The oil is extracted from the Citrus bergamia Risso's peel that contains highly therapeutic action compounds such as linalyl acetate, linalool and geraniol which effectively inhibit the growth of bacteria [11].

Currently, the main option for primary focal hyperhidrosis is a topical antiperspirant containing aluminium chloride [12]. The mechanism of action of the aluminium salt is to block the eccrine sweat gland and precipitate with mucopolysaccharides. The precipitation raises the pH value from neutral to basic which results in the damaging of epithelial cells along the duct then forming a plug that blocks the sweat output [13].

However, the current treatment only blocks the activation of the eccrine gland without addressing the issue of bacteria growth is caused by excessive sweat produced. The secretion of sweat enhances the growth of microbial flora, especially in closed areas such as the feet and armpits. Therefore, the formulation of an antiperspirant cream combining bergamot essential oil is believed to address both hyperhidrosis and bacterial overgrowth, thereby boosting patients' confidence in their daily social life.

## Materials and methods

### Formulation of antiperspirant lotion containing antibacterial agent

Emulsions of different compositions (F1 to F8) were prepared by using aluminium chloride (20 to 30%), bergamot essential oil (3 to 9%) and Tween 80 (3 to 9%). Oil and aqueous phases were prepared separately. The aqueous phase contains stock solution of aluminium chloride and water, while the oil phase contains bergamot essential oil and Tween 80. Phenonip was utilized as preservative. The stock solution of aluminium chloride and distilled water was prepared by the ratio of 1:1 (w/w). The aqueous phase and the oil phase were prepared separately in a water bath at 50°C and stirred on a hot plate. The oil phase was gradually added into the aqueous phase while stirring with an overhead stirrer (HS-100D, Daihan Laboratory) and continuously stirred for 3 hrs at 500 rpm speed. The sample was then further mixed with a high-shear homogenizer (Ultra-Turrax T25, IKA) at 11,000 rpm for 15 mins. The composition for each formulation (F1 to F8) is shown in Table 1.

### pH stability test

All emulsions were assessed using a digital pH meter (pH 2700, EUTECH). The pH of the emulsion was adjusted to a range of 4.0 to 5.5 was using 0.2M sodium hydroxide. The analysis of pH was carried out weekly for a month at different storage temperatures (4, 25 and 34°C). The

measurements for each sample were done in triplicate [14].

### Accelerated stability test

The centrifugation process was conducted using a centrifuge (Model 4000, KUBOTA). Each emulsion (F1 to F8) was placed in a test tube and centrifuged at 2,300 rpm speed for 15 mins. The stability of the emulsion was evaluated based on its phase appearance [14].

### Overtime stability test

All the formulated emulsion (F1 to F8) were placed and kept at different storage temperatures (4, 25 and 34°C) for 4 weeks. Physical observations and pH measurements were carried out weekly, with each measurement taken in triplicate [14].

### Freeze-thaw stability test

The formulated emulsion (F1) was incubated at different temperature cycles; (i) 4 and 25°C (ii) 25 and 35°C and (iii) 4 and 35°C for 24 hrs each. The study was carried out for 6 cycles. Physical observation and pH evaluation were recorded after the cycles finished [14].

### Disk diffusion assay

The disk diffusion analysis was carried out using *Staphylococcus epidermidis* (Gram positive bacteria) and *Escherichia coli* (Gram negative bacteria). Each bacteria colony was cultured from a bacteria stock for three days. The colonies were transferred into Mueller Hinton broth solution and incubated for 2 hrs at 37°C. The bacteria broth undergoes a turbidity test using spectrophotometer at a wavelength of 600 nm within 0.08 to 0.13 mg/L. The inoculation of plate was carried out by streaking the agar with the swab containing the inoculum. The surface was allowed to dry for 5 mins. Three disks with a diameter of 6 mm filled with 20 µL of test compound were placed on each plate; (i) pure bergamot oil (positive control) (ii) antiperspirant emulsion without bergamot oil (negative control) and (iii) formulated emulsions (samples). The plates were then incubated for 24 hrs at 37°C. The

inhibition zone was observed and measured using a ruler graduated to 0.5 mm [15].

### **Statistical analysis**

All analyses were carried out in triplicate and all data are shown as mean  $\pm$  standard deviation (n=3).

## **Results and discussion**

### **Accelerated stability analysis**

All formulated emulsions (F1 to F8) were found to be stable due to the absence of physical changes, phase separation and precipitation (Figure 1). Emulsion stability was determined based on the ratio of the total liquid phase volume that separated after a specified time of the centrifugation process to the volume of the sample [16]. The higher centrifugation speed and longer centrifugation time were able to accelerate the separation process. According to Kusumastuti et al. [17], the emulsification efficiency for centrifugation process increased to 97% at 3500 rpm speed for 15 mins.

The stability was achieved due to a proper method had been used during the emulsification process. The mixing process of the aqueous and oil phases was conducted using low and high-speed energy emulsification techniques to form a stable emulsion. Particle size, distribution of particle size, density between the dispersed and continuous phases, and chemical integrity of the dispersed phase are the factors that influence emulsion stability [18]. A fine emulsion can be produced from large particles that are broken down into smaller droplets by the application of mechanical energy [18]. In this study, high shear homogenizer was used to apply a high mechanical impact towards the mixture of both phases during the homogenization process. Therefore, a stable emulsion was proven to be achieved by this combination of emulsification methods.

### **pH stability analysis**

The emulsions withstanding both different conditions with a slight insignificant decrease

( $p < 0.05$ ) of pH. The results proved the ability of the emulsions to retain a stable system by maintaining its pH values (Figure 2). In addition, any topical application should not compromise the acidic pH of the natural human skin (pH 4.0 to 5.5) [19]. The acid surface of the skin is important for controlling the growth of skin microflora, maintaining a good skin condition, and forming an optimal structure of lipid barrier and stratum corneum homeostasis [20]. Hence, the slight change of the emulsion pH within the ideal range for skin is acceptable.

### **Emulsion physical analysis**

Table 2 shows the physical and chemical stability of the emulsions were examined through a long-term stability study for four weeks. The study has been carried out to determine the recommended storage condition and the shelf-life of the emulsions to ensure its safety and effectiveness. Stability testing aims to provide information on the changes of the emulsion's quality over a longer period under the influence of certain environmental factors such as temperature, humidity, and light. The test period is related to the stability of the product which should be long enough to prove no degradation has occurred [21].

### **Effect of bergamot essential oil content**

F1, F2 and F3 were varied in terms of the bergamot essential oil content (3, 6 and 9% respectively) with constant amount of tween 80 (6%) and aluminium chloride (25%). F1 was stable at all storage temperatures with no sign of phase separation. However, at 34°C storage temperature, layer separation was observed in F2 and F3. In this study, increased bergamot essential oil content directly decreased the amount of water per formulation. The water content and the temperature could affect the stability of the emulsion [22]. A study discovered by Raya et al. [23] proved that the decrease in water content decreases the rate of emulsification. Therefore, this explains the instability of F2 and F3 compared to the stable F1 that has the highest water content compared to F2 and F3. The

stability of the emulsion is depending on the densities between oil phase and aqueous phase, as well as the unfavourable contact between molecules in both phases [8].

According to Goodarzi and Zendeboudi [22], the phase ratio can alter the viscosity of the emulsion and thus, the coalescence rate rapidly occurs due to the increase of flow resistance between the particles in the emulsions. Concerning the storage temperature, high temperature increases the demulsification process by increasing the water particle collisions. The viscosity was observed to be decreased by the increasing temperature (34°C). Hence, the stability of the F2 and F3 were reduced due to the high ratio of oil/water phase and high storage temperature. Moreover, the observation of physical appearance for F1, F2 and F3 shows that there was no growth of mould was observed in this stability analysis. This proved the emulsions were formulated with a sufficient amount of preservative and successfully inhibits the growth of mould.

At high temperature (34°C), F2 to F8 turned into a light-yellow colour from the initial bright yellow colour, except for F1 that remained its initial phase colour. Exposure to high temperatures has a crucial impact on the essential oil integrity [24]. Chang et al. [25] mentioned that linalool, a thermal sensitive compound presents in bergamot essential oil, undergoes decomposition of chemical structure through the dehydroxylation process. Alteration of chemical stability can cause instability of the emulsion causing destabilization processes such as physical changes, colour changes, and oil and aqueous phase separation [26]. Hence, the physical instability of the emulsions is also being affected by the degradation of the chemical structure of bergamot essential oil causes by high temperature storage.

#### **Effect of Tween 80 content**

F4, F2 and F5 were formulated with various content of tween 80 (3, 6, and 9%, respectively)

with a constant amount of bergamot essential oil (6%) and aluminium chloride (25%). These three emulsions were found to be stable at 4 and 25°C storage temperatures, but not stable at higher temperatures. Theoretically, as the content of the surfactant increased, the stability of the emulsion increased too. Henriquez stated that the high concentration of surfactant in the aqueous phase allows the saturation of surfactant molecules on the interface and then accumulates on the particle in the emulsion [27].

At some point, the surfactant molecules start forming micelles where the hydrophobic side is removed from the aqueous environment. However, the high temperature allows the process of reverse micelles where it increases the disproportionation process of the surfactant molecules which increases the particle screening and decreases the maximum particle mobility [28]. The emulsion instability forms a floating droplet on the surface, cohesion between droplets, and finally leads to creaming and separation [29]. Even though the emulsion was formulated with a high concentration of surfactant, the high temperature can alter the process of forming a micelle which results in instability of the formulations.

#### **Effect of aluminium chloride content**

F6, F7, and F8 were formulated with various contents of aluminium chloride (20, 25, 30%, respectively) with constants amount of bergamot essential oil (10%) and tween 80 (6%). Best formulation was possessed by F7 and F8 where the emulsions were stable at both 4 and 25°C. In contrast with F6, the emulsion was not stable even at 25°C. This finding could be explained by the effect of aluminium chloride presented in each emulsion.

According to Clark [30], a solution of aluminium chloride (1 mol/dm<sup>3</sup>) has a pH reading of 2 to 3. Emulsion droplets were reported to be more stable under more acidic and saline conditions. Due to the increases of the emulsifier ability to

absorb on the oil/water interface and produce smaller emulsion droplets during the preparation process of the emulsions [31]. In this study, F6 contain the least amount of aluminium chloride than F7 and F8 which directly caused its less acidic system. Therefore, it was proven that higher physical stability can be achieved by formulating an acidic emulsion system.

### Freeze-thaw stability analysis

From the stability evaluation studies, F1 was selected to be the optimum composition with the greatest stability among the formulated emulsions. F1 was stored in two-cycle of freeze and thaw analysis under different investigation conditions: (i) 4 and 25°C (ii) 25 and 34°C (iii) 4 and 34°C. The emulsion was examined for a week under extreme temperature changes and were proven to own a good stability system against deterioration due to the absence of physical changes (**Figure 3**). In addition, the stability of emulsion has also been supported by insignificant small changes ( $p < 0.05$ ) of pH within the acceptance range of skin use (4.0 to 5.5).

The freeze-thaw stability test is used to analyse the stability of emulsion under various conditions. The cyclic temperature stress was designed to provide a knowledge of the product to mimic likely with the conditions in marketplace storage [21]. Magnusson et al. mentioned that the freeze-thaw stability is mainly collated with the composition and crystallization behaviour of oils and the freezing conditions of the emulsion [32]. Hence, the emulsion was confirmed to have an acceptable stability system when encounter extreme changes in storage conditions.

### Disk diffusion analysis

Table 3 shows the disk diffusion analysis of *Staphylococcus epidermidis* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria) using the disk diffusion method. According to Reller et al. (2009), the main reasons for the testing are to determine the resistance of possible drugs towards common

pathogens and to ensure susceptibility to drugs of choice for certain infections [33]. Linalool is a component that contains in bergamot essential oil that seizes anti-bacterial properties [34]. The acidity of the bergamot essential oil can provide antibacterial properties due to the high content of ascorbic acid and citric acid. The pH of the bergamot juice is found to be lower than pH 3.05 but similar to or higher than pH 2.43 [35]. Bacteria have high sensitivity towards low pH since the increase of bergamot essential oil's hydrophobicity will raise the rate of dissolution of the membrane lipid's target bacterium [36].

From the result, the Gram-positive bacteria were more susceptible compared to Gram-negative bacteria. Both positive (bergamot essential oil) and negative control (emulsion without bergamot essential oil) exhibited smaller inhibition zones than obtained by the emulsion sample. The inhibition zone for the (positive control) was smaller than the emulsions (F1 to F8). The zone of inhibition of emulsions (F1 to F8) for Gram-positive bacteria indicates that the combination of the aluminium chloride as the active ingredient in antiperspirants emulsion with the bergamot essential oil was able to boost its ability to inhibit the *Staphylococcus epidermidis* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). The largest inhibition zone caused by the formulated emulsions has proven the efficacy of incorporating bergamot essential oil into the formulation.

The resistance mechanism creates by the bacteria protects them from antibacterial activity of bergamot essential oil. The low resistance of the bacteria to the bergamot essential oil is due to the lack of an outer membrane found in Gram-negative bacteria, but the thick layer of the peptidoglycan layer that surrounds the outer membrane provides the survival mechanism for the bacteria to live in harsh environment [37]. Peptidoglycan layer of the bacteria is the best target for inhibition activity for antibacterial



agents by altering the peptidoglycan synthesis [38].

Lazarotta et al. [11] stated that the Minimal Inhibitory Concentration (MIC) of the bergamot essential oil for Gram-negative bacteria is 125 µg/mL. Some negative bacteria especially *Escherichia coli* have developed resistance to most available antibacterial agents [39]. The lipopolysaccharide on the bacteria wall is able to block the permeation of hydrophobic properties of bergamot essential oil and avoids the accumulation of the oil in the target cell membranes [39]. The complexation of the cell wall increases the survival rate of the bacteria toward the antibacterial activity of the bergamot essential oil.

### **Conclusions**

The result achieved in the present study shows that F1 is the optimum formulation for antiperspirant lotion containing bergamot essential oil as an antibacterial agent. It possessed good stability at all storage temperatures (4, 25 and 34°C), no phase separation, no precipitation, no colour change, and minimal pH changes. The freeze-thaw stability showed great stability throughout the six cycles of storage in various temperature conditions, demonstrating an ideal property for shipping and transportation of the emulsion. Disk diffusion analysis proved that the addition of bergamot essential oil into the

antiperspirant formulation was able to boost the ability to inhibit the growth of *Staphylococcus epidermidis* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). Unlike the pure bergamot essential oil, it has failed to inhibit the growth of *Escherichia coli* due to the presence of lipopolysaccharide that blocks the permeation of the antibacterial agents. Overall, the results obtained in this study pointed out the successful development of an antiperspirant lotion containing bergamot essential oil, which might be used as an emulsion that blocks the production of sweat and inhibits the growth of bacteria.

### **Conflicts of interest**

The authors have declared that no competing interests exist.

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### **Authors' Contribution**

SHM: Conceived the research, conducted the analysis, and drafted the manuscript.

NII: Study the theory and performed the analysis.

FNR: Verified the analytical methods.

All authors discussed the results and contributed to the final manuscript.

Table 1. Composition of antiperspirant lotion with bergamot essential oil.

Ingredients	Formulation composition (%)							
	F1	F2	F3	F4	F5	F6	F7	F8
Aluminium chloride	25	25	25	25	25	20	25	30
Bergamot essential oil	3	6	9	6	6	10	10	10
Tween 80	6	6	6	3	9	6	6	6
Phenonip	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Table 2. Physical observations of F1 to F8 after 4 weeks of storage.

After 4th weeks of observation	Storage temperature	F1	F2	F3	F4	F5	F6	F7	F8
Phase separation	4°C	X	X	X	X	X	X	X	X
	25°C	X	X	X	X	X	✓	X	X
	34°C	X	✓	✓	✓	✓	✓	✓	✓
Colour changes	4°C	X	X	X	X	X	X	X	X
	25°C	X	X	X	X	X	X	X	X
	34°C	X	✓	✓	✓	✓	✓	✓	✓
Presence mould	4°C	X	X	X	X	X	X	X	X
	25°C	X	X	X	X	X	X	X	X
	34°C	X	X	X	X	X	X	X	X

✓: Phase separation/ colour changes/ Presence mould is visible.

X: Phase separation/ colour changes/ Presence mould is not visible.

Table 3. Disk diffusion data of emulsions (F1 to F8) on *Staphylococcus epidermidis* and *Escherichia coli*.

Tested bacteria	Tested emulsion	Inhibition zone (mm)		
		Pure bergamot essential oil	Control emulsion	Formulated emulsion
<i>Staphylococcus epidermidis</i>	F1	11±0.16	26±0.12	29±0.17
	F2	10±0.12	25±0.17	30±0.16
	F3	10±0.12	26±0.12	32±0.17
	F4	11±0.13	27±0.16	30±0.12
	F5	10±0.16	26±0.16	28±0.17
	F6	9±0.17	25±0.12	29±0.17
	F7	9±0.12	26±0.15	27±0.17
	F8	9±0.17	26±0.10	26±0.17
<i>Escherichia coli</i>	F1	-	21±0.16	23±0.21
	F2	-	31±0.22	32±0.16
	F3	-	21±0.21	23±0.16
	F4	-	21±0.19	21±0.12
	F5	-	20±0.12	21±0.16
	F6	-	27±0.17	28±0.12
	F7	-	20±0.21	21±0.22
	F8	-	26±0.17	28±0.17

- : No inhibition zone

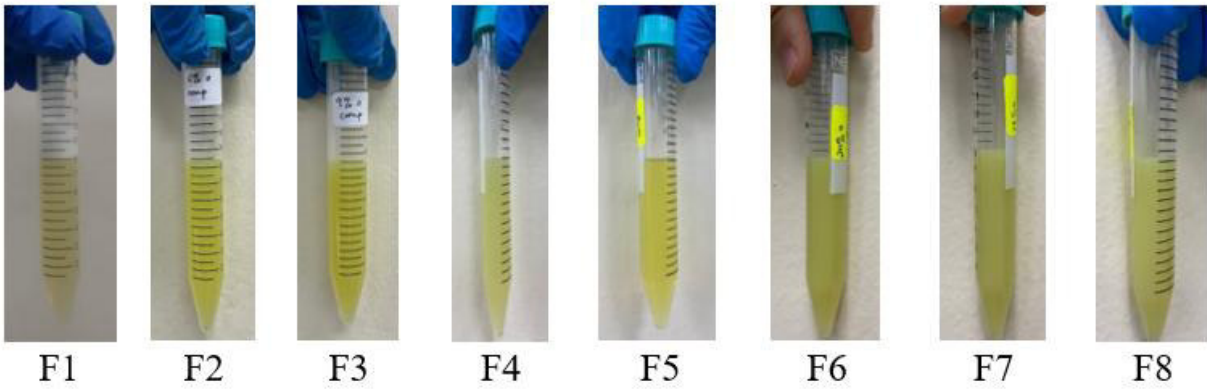


Figure 1. The physical evaluation of emulsions (F1 to F8) consisting of different composition of bergamot essential oil, aluminium chloride and tween 80 after the centrifugation process.

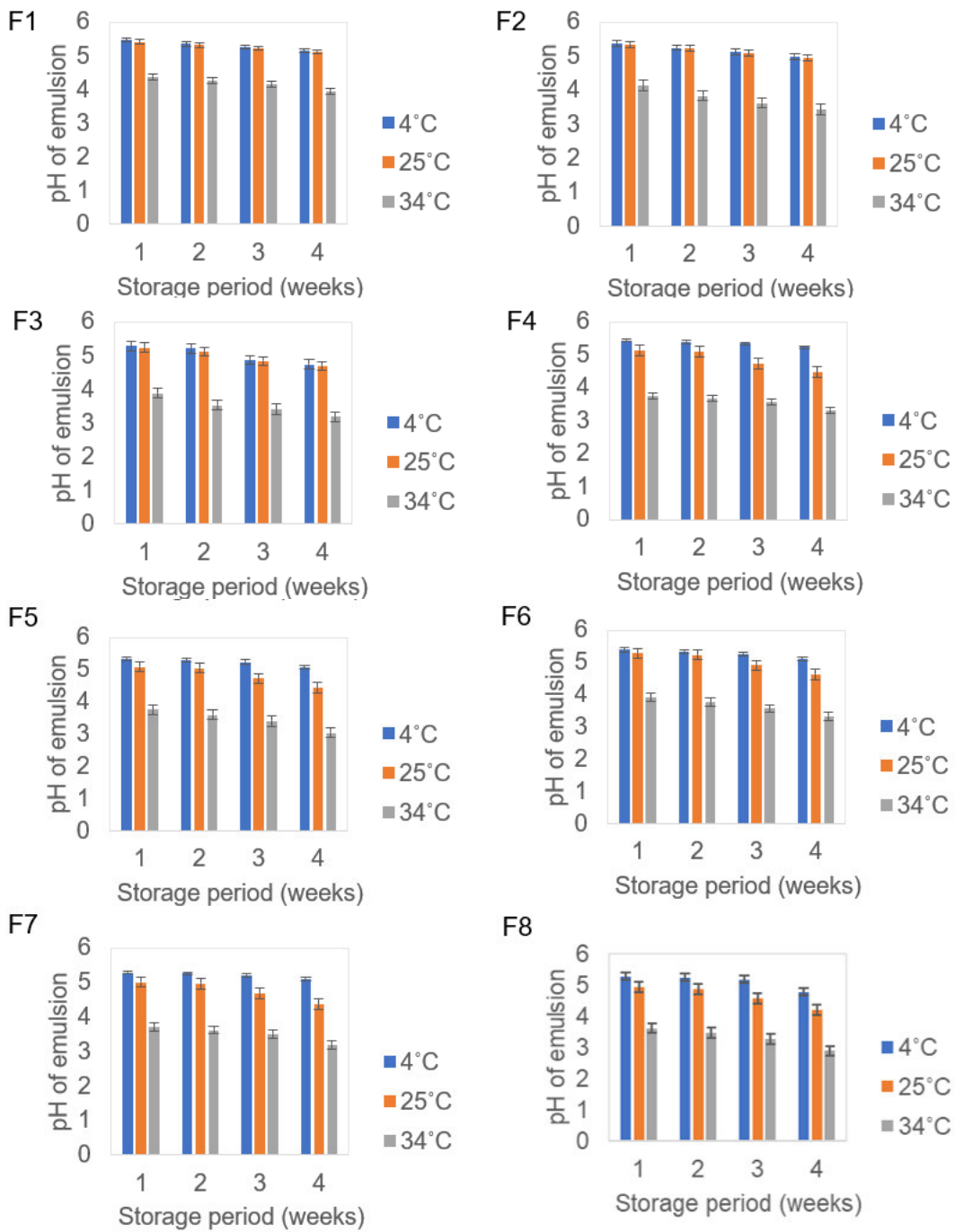


Figure 2. Weekly pH evaluation of emulsions (F1 to F8) for 1mth of storage at different temperatures: 4, 25 and 34°C.

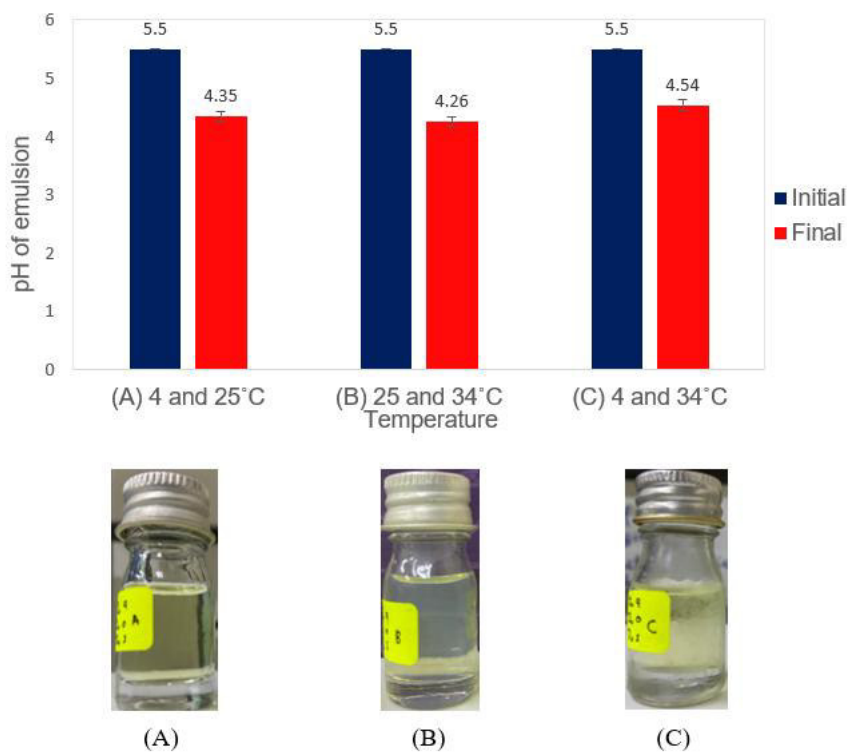


Figure 3. The pH value and physical observations for formulated emulsion after 6 cycles in freeze-thaw analysis.

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ORIGINAL ARTICLE

## Awareness, Knowledge and Attitudes Towards Safe Antibiotic Use among Outpatient Visitors at a Teaching Hospital in Malaysia.

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### Abstract

**Introduction:** Antimicrobial resistance has emerged as a significant issue largely due to the misuse of antibiotics in treating bacterial infections since their introduction. Misuse of antibiotics includes failure to finish the full course and prematurely stopping their antibiotic treatment after symptoms improve. Although antibiotics cannot be self-prescribed in Malaysia, misuse still occurs among consumers. **Purpose:** This study aimed to assess the level of awareness, knowledge and attitude (AKA) on safe antibiotic use among outpatient visitors of a teaching hospital in Pahang, Malaysia. The association between sociodemographic background and AKA score was also explored to identify the knowledge gap within various backgrounds. **Methods:** A total of 206 participants were recruited for this study. The participants were approached at several outpatient departments in a teaching hospital using convenient sampling from May until June 2022 and completed a set of validated questionnaires. The data were analysed using SPSS. **Results:** Only 35.4% of participants obtained a high score in knowledge, while the majority scored moderately. Among the 206 participants, 51.0% had poor awareness of safe antibiotic use. More than half of the participants (63.1%) exhibited a positive attitude towards antibiotic consumption. Education level and medical background were shown to be statistically significant to AKA scores. **Conclusion:** This study highlights that despite moderate knowledge and poor awareness of safe antibiotic use among hospital attendees, a positive attitude towards antibiotics was observed. It is essential to implement public educational campaigns that address knowledge gaps and misconceptions to improve antibiotic safety among the population.

**Keywords:** antibiotic, antimicrobial resistance, attitude, awareness, knowledge.

## Introduction

Antibiotics are one of the most commonly prescribed medications to treat a wide range of bacterial and fungal infections [1]. In Malaysia, antibiotics are not recognised as over-the-counter (OTC) drugs. Nonetheless, the national surveillance program continues to keep us informed about emerging and newly identified resistance threats [2]. This raises questions about why the antibiotic resistance rate in our country is still increasing. Antibiotic resistance represents a significant global challenge in healthcare, complicating the treatment of diseases that are commonly manageable. The annual death toll linked to antibiotic and other antimicrobial resistance (AMR) is currently around 7000,000, with projections indicating it could reach 10 million by 2050 if antibiotic use is not controlled [3].

Since their introduction, the misuse of antibiotics in treating bacterial infections has led to the emergence of AMR as a major concern. Misuse includes not finishing the full course of treatment, stopping medication when feeling well, saving some for future use, skipping doses, taking double doses to compensate for the missed ones, and using antibiotics as painkillers [4]. These behaviours indicate a lack of awareness regarding the safe use of antibiotics in the community, which has been a highly contributing factor in the increasing number of antibiotic-resistant cases worldwide.

Evidence shows that a lack of consumer knowledge of correct antibiotic consumption interferes with the government's efforts to curb AMR. Knowledge is the most important aspect of combating misinformation, and focusing on measuring the population's knowledge, awareness, and attitude will provide a clearer picture of the consumers' understanding of antibiotics. Outpatient visitors to healthcare facilities play a pivotal role in the fight against antibiotic resistance, as they are considered healthcare seekers in the hospital setting and may be more inclined to use antibiotics, along with other medications. This study aimed to explore the level of awareness, knowledge, and attitude

(AKA) regarding safe antibiotic use among outpatient visitors of a tertiary hospital in Kuantan, Pahang. The association between AKA score and sociodemographic characteristics, the correlation between knowledge and awareness score, and the correlation between knowledge and attitude score were investigated. The findings will provide valuable insights into the current awareness levels among patients, allowing the teaching hospital to identify areas for potential intervention among healthcare workers and within the hospital environment, thereby enhancing patient care and educational efforts. By educating patients, the hospital not only improves individual health outcomes but also contributes to the broader community's understanding of antibiotic use and resistance.

## Materials and methods

This quantitative, cross-sectional study was conducted between May and June 2022. A total number of 206 participants were recruited using a convenient sampling method. The participants were approached at several outpatient departments at a teaching hospital. Eligible participants were at least 18 years old, able to understand and read Bahasa Malaysia, and visiting the hospital for various purposes. Those who agreed to take part in the study signed a consent form.

To measure the intended variables, this study used a set of validated questionnaires by Neni et al. [5] to explore the participants' level of knowledge, awareness, and attitude towards safe antibiotic use. The questionnaire consisted of 4 parts. Part A included sociodemographic questions, Part B assessed knowledge with 12 items, Part C evaluated awareness with 6 items, and Part D examined attitude with 9 items. The questionnaire was translated from English to Malay using back-to-back translations and validated by a qualified Malay language educator. Prior to the main study, a pilot study was conducted, yielding a Cronbach's Alpha coefficient of 0.82. The data obtained were

analysed using Statistical Package for Social Science (SPSS) version 27. Descriptive statistical analysis was used to measure the frequency and percentage of variables. The Mann-Whitney test was applied to analyse two categorical outcome variables, namely gender and medical background, whereas the Kruskal-Wallis test was employed to examine associations involving more than two categorical outcome variables, including age category, marital status, education level, and household income. A P-value of less than 0.05 was considered statistically significant, and 95% confidence intervals (CIs) were reported for all relevant outcomes.

The ethical approval for this study was obtained from the IIUM Research Ethic Committee (IREC 2022-KON/54). Each participant has been provided with an information sheet informing them about the objectives and confidentiality of the study before obtaining consent to participate.

## Results

Of the total 206 respondents, 83 (40.3%) were male and 123 (59.7 %) were female. Their ages ranged from 18 to 72 with a mean age of 33 (SD=13.94). Among them, 29.9% were students, 49% were employed, and 21.4% were unemployed or retired. Regarding the education level, 72.3% reported having tertiary education, 26.7% had graduated from secondary school, and only 1% had completed primary education. Half of the participants (54.4%) reported living in the city, while the other half resided in suburban areas. In terms of household income, 78.6% of respondents came from families earning less than RM 4,850 monthly. Additionally, 30.6% of participants recorded that they had a medical background, while 69.4% had no prior involvement in the healthcare field through their career or education.

### Awareness, Knowledge and Attitude (AKA) Score and Level

Based on three domains in AKA, the individual and total median scores of Knowledge,

Awareness, and Attitude among the outpatient visitors were presented in Table 1 . Details of the respondents' answers concerning their knowledge, awareness, and attitude are presented in Table 2.

### AKA Score Category

From the score, the results are divided into three categories Low, Moderate, and High level of AKA. The findings are presented in Table 3. Overall, it can be said that most of the respondents have moderate to high levels of awareness, knowledge, and attitude regarding antibiotic usage.

### Association between AKA Score and Sociodemographic data

Table 4 below shows the association between sociodemographic characteristics (gender, education level, marital status, household income, medical background) and total AKA score. For age, the test indicated no significant association between different age groups and AKA scores ( $P = 0.397$ ). Similarly, there was no statistically significant difference between gender categories and AKA scores ( $P = 0.082$ ). However, concerning education level, the test demonstrated a significant difference in total AKA scores across different education levels ( $X^2 = 25.71, P < 0.001$ ), with mean rank scores varying notably. The significant difference is between participants with secondary education (Mdn:13) and tertiary 1 (diploma or bachelor's degree) (Mdn:18) and tertiary 2 (master's degree and doctorate) (Mdn:18) (Figure 1). The effect size for this analysis was calculated using Eta-squared ( $\eta^2$ ) and found to be  $\eta^2 = 0.112$ , indicating a medium effect of education level on AKA scores. Likewise, a medical background revealed a significant difference in AKA scores ( $U = 2157, P < 0.001$ ), as they scored higher (Mdn: 21) than people who did not have a medical background (Mdn:15) (Figure 1). The effect size was calculated using the biserial correlation ( $r$ ) yielding an effect size of 0.521, which indicates a large effect size. Conversely, marital status and

household income did not exhibit a significant difference in AKA scores ( $P > 0.05$ ).

### **Correlation between Knowledge, Awareness and Attitude**

The Spearman correlation test for the knowledge and awareness domain indicates a significant correlation between the two variables ( $r_s(204) = 0.695$ ,  $P = 0.001$ ). The positive value of the correlation coefficient between the knowledge score and awareness score signifies a direct and strong association as the coefficient value is more than 0.6. Similarly, in this study, the Spearman correlation coefficient revealed a positive correlation between knowledge and attitude score ( $r_s(204) = 0.461$ ,  $P = 0.001$ ). However, with a coefficient value below 0.5, this association is considered moderate.

### **Discussion**

#### **Knowledge of safe antibiotic use**

In this study, 66% of the visitors knew that penicillin is an antibiotic but not aspirin (43.7%) and paracetamol (72.8%). This shows that half of the visitors did not know the correct indication for aspirin. However, paracetamol is a more frequently used over-the-counter drug here in Malaysia compared to aspirin, so its use is well understood. Additionally, 79.6% knew that antibiotics are used to treat bacterial infections, while 64.6% also believed that antibiotics are useful for viral infections. This explains why some patients still request for antibiotics when visiting clinics for upper respiratory tract infections, common cold, or viral fevers [6]. The result is consistent with a study conducted in Boyolali, Indonesia, where 73.12% of the respondents answered that antibiotics could be used to treat infections that are caused by viruses. Overall, the knowledge level of most hospital visitors in this study was categorized as moderate. Similar findings were reported by Kong et al. [8] and Pauzi et al. [9], who found that the level of knowledge of antibiotics among the majority of adult respondents was moderate. Another study

conducted by Neni et al. [5] on high school students, discovered that adolescents aged 13, 14, and 16 also reported moderate levels of knowledge on antibiotics. The moderate knowledge level observed across different studies indicates a broader trend in public understanding of healthcare topics. Given that this moderate level of knowledge towards antibiotics begins as early as secondary school, there is an opportunity for targeted public health education initiatives. By focusing on educating this demographic on safe antibiotic use, there is potential for them to carry this knowledge into adulthood and serve as agents of change within their families and communities. However, previous studies conducted among the general adult population in Kuantan [10] and across Malaysia [11] revealed that a higher proportion of respondents demonstrated good knowledge regarding antibiotics and their usage. The discrepancies observed between these findings and our study may be attributed to the different tools employed to assess knowledge, as well as other contextual factors. Notably, the study among the general public in Kuantan required participants to have prior awareness of antibiotic agents [10], which likely contributed to the elevated reported levels of knowledge and usage. In contrast, our study encompasses a more diverse demographic, including individuals who may lack such awareness.

Furthermore, the population reported in the study by Bhatt et al. (2023) focused on populations residing in the central region of Malaysia, with only a small representation from the east coast, where our study population is [11]. Additionally, their sample predominantly consisted of urban residents, whereas our study reflects a balanced representation of both urban and suburban populations. The geographic differences may thus influence antibiotic knowledge levels, as suggested by findings from another study, which indicates that individuals in urban areas tend to exhibit greater knowledge of antibiotic use [12].

#### **Awareness of Safe Antibiotic Use**

From the study, 48.5% of the visitors had heard about the “Antibiotic Resistance” issue, whereas

only 30.6% had ever talked or discussed about it. While more than half (58.7%) of the participants knew that when a person has bacteria resistant to antibiotics, the infection will be challenging to treat, only 18.9% (n=39) of the participants answered correctly when asked if antibiotic resistance is only a problem for people who take antibiotics regularly. Previous studies conducted in Nigeria [13] and Malaysia [5] also reported that respondents believed antibiotic resistance is only a problem for those who take antibiotics regularly. This shows that most people do not know that antibiotic resistance does not primarily occur due to increased antibiotic consumption, but rather from the misuse of prescribed antibiotics.

There is a significant difference in awareness scores between education levels, with participants who have tertiary level of education scoring higher compared to those without tertiary education. Exposure to issues related to antibiotic resistance during tertiary education and literacy towards complicated medical terms (e.g. antibiotic resistance) might be the potential factors in the difference in awareness level and score. This is supported by an article published by Karuniawati et al. [7] which discussed the inadequacy of information acquired during schooling about antibiotics will impact the level of awareness. Overall, the participants in this study showed moderate awareness of safe antibiotic use.

#### **Attitude towards Safe Antibiotic Use**

Most hospital attendees in the current study exhibited a positive attitude towards antibiotic use, consistent with findings from previous studies [9,10]. The majority (85.4%) of the respondents answered that they would only take antibiotics when prescribed by a doctor, which is consistent with other studies carried out in Nigeria (86%) [13] and two other studies conducted in Malaysia, which reported rates of 82.4% and 96.5% respectively [5,8]. This is a good sign that the participants know that antibiotics are not over-the-counter drugs and cannot be self-prescribed in Malaysia. Even

though 45.1% of respondents in the current study indicated that they would stop taking antibiotics when already feeling well, 70.9% understood that they should finish their antibiotics even though their symptoms disappeared. This suggests a discrepancy between knowledge and behaviour regarding antibiotic use among respondents. Despite the majority of respondents being aware of the correct antibiotic usage protocol, some may still be inclined to prematurely stop their antibiotic treatment. Possible explanations for this observation are that there might be a misconception about the necessity of completing the course, a lack of understanding about its connection to antibiotic resistance, or even convenience. This highlights the need for further education and communication efforts not only to inform individuals about proper antibiotic usage but also to emphasise the broader implications of antibiotic resistance.

#### **Association between AKA Level and Sociodemographic Characteristics**

Education level showed a significant association with AKA level. Kong et al. [8] and Oh et al. [14] also reported that education level has a significant role towards knowledge level on antibiotics. People with higher education levels usually secure more general knowledge compared to those who only received lower formal and informal education which is probably due to exposure in the tertiary level education. Apart from that, having a medical background also impacts AKA score, particularly in the knowledge and awareness domains. This is logical, as individuals whose work or studies are related to healthcare tend to acquire information or insight about antibiotics. A study conducted by Suaifan et al. [15] also shows similar findings, where medical students scored better than non-medical students in their knowledge of antibiotics. The age categories did not show a significant difference in AKA scores. In contrast to other studies that suggest older individuals tend to have more knowledge about antibiotics, our findings do not support this assumption. The discrepancy

may be attributed to the similar characteristics of health-seeking behaviour shared by the population of this study. The purpose of the hospital visit was to have an appointment with the doctor, resulting in most of them having acquired similar knowledge regarding antibiotics compared to the general public outside the hospital. In terms of gender, there is also no significant difference in AKA scores between male and female participants, aligning with the notion that gender does not create a significant gap in general knowledge. Therefore, there is no necessity to tailor educational interventions on antibiotic usage based on gender.

### **Correlation between Knowledge, Awareness and Attitude of Visitors**

We have reported that the results of correlation analysis demonstrated a significant positive correlation between knowledge and awareness, as well as knowledge and attitude. The correlation coefficient indicates a strong positive correlation, suggesting that visitors with higher knowledge scores are more likely to have higher scores in the awareness domain. However, in a study conducted on high school students in Malaysia, the awareness was not correlated to any domain and only knowledge and attitude were significantly associated [5]. Karuniawati et al. [7] also noted that knowledge alone is not sufficient to change behaviour, but it plays a crucial role in shaping beliefs and attitudes toward certain behaviours. In our study, the correlation between knowledge and attitude is moderate.

Regardless of having low awareness and moderate knowledge, most visitors exhibit a positive attitude towards antibiotic use. This suggests that proper education can effectively enhance the correct use of antibiotics among hospital visitors, thereby helping to prevent the adverse consequences of antibiotic misuse, particularly antimicrobial resistance. As for public intervention, future efforts could focus on developing targeted educational strategies such as active public health campaigns and educational programs to address specific knowledge gaps or

common misconceptions about antibiotics. Engaging the community through partnerships with local organisations, healthcare providers and related academic institutions can amplify the impact of these educational efforts.

Despite the valuable insights gained from this study, one limitation of this study is the sampling method, which utilised convenient sampling and focused exclusively on individuals attending outpatient clinics at the hospital. While this approach provides valuable insights specific to the hospital context, it may not fully represent the broader local population, limiting the generalisability of the findings. However, the study population consists of diverse characteristics, with approximately equal representation from both urban and suburban areas. The wide age range also ensures a balanced and varied demographic, allowing for a more comprehensive understanding of awareness, knowledge, and attitudes toward antibiotic use across different segments of the community.

### **Conclusion**

This cross-sectional study aimed to assess the level of knowledge, awareness and attitude toward safe antibiotic use among outpatient visitors in a tertiary hospital in Pahang, Malaysia. The study found that overall knowledge among public visitors regarding antibiotics was moderate, while awareness of safe antibiotic use was poor among half of the respondents. Despite this, the attitude towards antibiotics was mostly positive, possibly attributed to routine health education provided by healthcare professionals emphasising the importance of completing the full course of antibiotics. To enhance antibiotic safety, future public interventions should focus on targeted educational campaigns to address knowledge gaps and misconceptions, alongside partnerships with local organisations and healthcare providers. It is hoped that these findings will provide insight into the current perceptions and behaviours of the public regarding safe antibiotic usage.



Table 1. Median Score of each domain and total AKA score.

<b>Variable</b>	<b>Median (IQR)</b>
<b>Knowledge score</b>	7.00 (4)
<b>Awareness score</b>	2.00 (3)
<b>Attitude score</b>	7.00 (2)
<b>Total AKA score</b>	16.00 (9)

Table 2. Knowledge, Awareness and Attitude Answers Distribution

<b>Questions</b>	<b>N (%)</b>	
	<b>Correct (%)</b>	<b>Wrong (%)</b>
<b>Knowledge</b>		
Penicillin and amoxicillin are antibiotics. (Yes)	136 (66.0)	70 (34.0)
Aspirin is an antibiotic. (Yes)	90 (43.7)	116 (56.3)
Paracetamol is an antibiotic. (No)	150 (72.8)	56 (27.2)
Antibiotics are useful for bacterial infections (e.g., UTIs). (Yes)	164 (79.6)	42 (20.4)
Antibiotics are useful for viral infections (e.g. flu). (No)	73 (35.4)	133 (64.6)
Antibiotics can reduce stress. (No)	105 (51.0)	101 (49.0)
Antibiotics can kill 'good bacteria' present in humans. (Yes)	61 (29.6)	145 (70.4)
It is okay to stop taking antibiotics when symptoms improve. (No)	116 (56.3)	90 (43.7)
Taking fewer antibiotics than prescribed is healthier than taking the full course. (No)	135 (65.5)	71 (34.5)
Antibiotics can cause allergic reactions. (Yes)	132 (64.1)	74 (35.9)
Antibiotic resistance is a phenomenon whereby a bacterium loses its sensitivity to an antibiotic. (Yes)	133 (64.6)	73 (35.4)
Misuse of antibiotics can lead to a loss of sensitivity of an antibiotic to a specific pathogen. (Yes)	145 (70.4)	61 (29.6)
<b>Awareness</b>	<b>Correct (%)</b>	<b>Wrong (%)</b>
Have you ever heard about antibiotic resistance? (Yes)	100 (48.5)	106 (51.5)
In particular, have you discussed the problem of antibiotic resistance in class? (Yes)	63 (30.6)	143 (69.4)
Antibiotic resistance occurs when your body becomes resistant to antibiotics and they no longer work as well. (Yes)	129 (62.6)	77 (37.4)
If bacteria are resistant to antibiotics, it can be very difficult or impossible to treat the infection they cause. (Yes)	121 (58.7)	85 (41.3)
Antibiotic resistance is only a problem for people who take antibiotics regularly. (No)	39 (18.9)	167 (81.1)
Bacteria which are resistant to antibiotics can be spread from person to person. (Yes)	68 (33.0)	138 (67.0)
<b>Attitude</b>	<b>Correct (%)</b>	<b>Wrong (%)</b>
Do you usually take antibiotics for nausea? (No)	177 (85.9)	29 (14.1)
Do you usually take antibiotics for fever? (No)	104 (50.5)	102 (49.5)
Do you usually stop taking antibiotics when you start feeling better? (No)	113 (54.9)	93 (45.1)

Do you only take antibiotics when prescribed by a doctor? (Yes)	181 (87.9)	25 (12.1)
Do you keep leftovers at home because they might be useful in the future? (No)	152 (73.8)	54 (26.2)
Do you use leftover antibiotics when you have a cold, sore throat, or fever without consulting a doctor? (No)	172 (83.5)	34 (16.5)
Do you buy antibiotics without a prescription? (No)	176 (85.4)	30 (14.6)
Have you ever started an antibiotic therapy after simply calling a doctor, without a proper medical examination? (No)	182 (88.3)	24 (11.7)
If symptoms improve before the full course of antibiotics is completed, you can stop taking them. (No)	146 (70.9)	60 (29.1)

Table 3. AKA Score Category Distribution

<b>Variable</b>	<b>Frequency (n)</b>	<b>Percentage (%)</b>
<b>Low</b>	27	13.1
<b>Moderate</b>	99	48.1
<b>High</b>	80	38.8

Table 4. Association between AKA Score and Sociodemographic Background.

Variables	Statistics		
	N (%)	Mean Rank	P-value
<b>Age</b>			0.397
18-30	120 (58.3)	108.05	
31-59	70 (34.0)	98.41	
>60	16 (7.8)	91.63	
<b>Gender</b>			0.082
Male	83 (40.3)	94.71	
Female	123 (59.7)	109.43	
<b>Education Level</b>			<0.001*
Primary	2 (1.00)	83.25	
Secondary	55 (26.7)	69.17	
Tertiary 1 (Diploma and Bachelor's degree)	130 (63.1)	115.45	
Tertiary 2 (Master's degree and doctorate)	19 (9.2)	121.26	
<b>Marital Status</b>			0.060
Single	97 (47.1)	106.76	
Engaged	13 (6.3)	107.77	
Married	90 (43.7)	103.67	
Divorced	6 (2.9)	39.00	
<b>Household Income</b>			0.453
<RM 4,580	162 (78.6)	100.79	
RM 4,581 – 10,970	34 (16.5)	114.09	
>RM 10,971	10 (4.9)	111.35	
<b>Medical background</b>			<0.001*
Yes	63 (30.6)	140.76	
No	143 (69.4)	87.08	

Table 5. Correlation between knowledge with awareness and attitude.

Variables	Knowledge	
	<i>rs</i>	P-value
<b>Awareness</b>	0.695	0.001
<b>Attitude</b>	0.461	0.001

*rs* = correlation coefficient

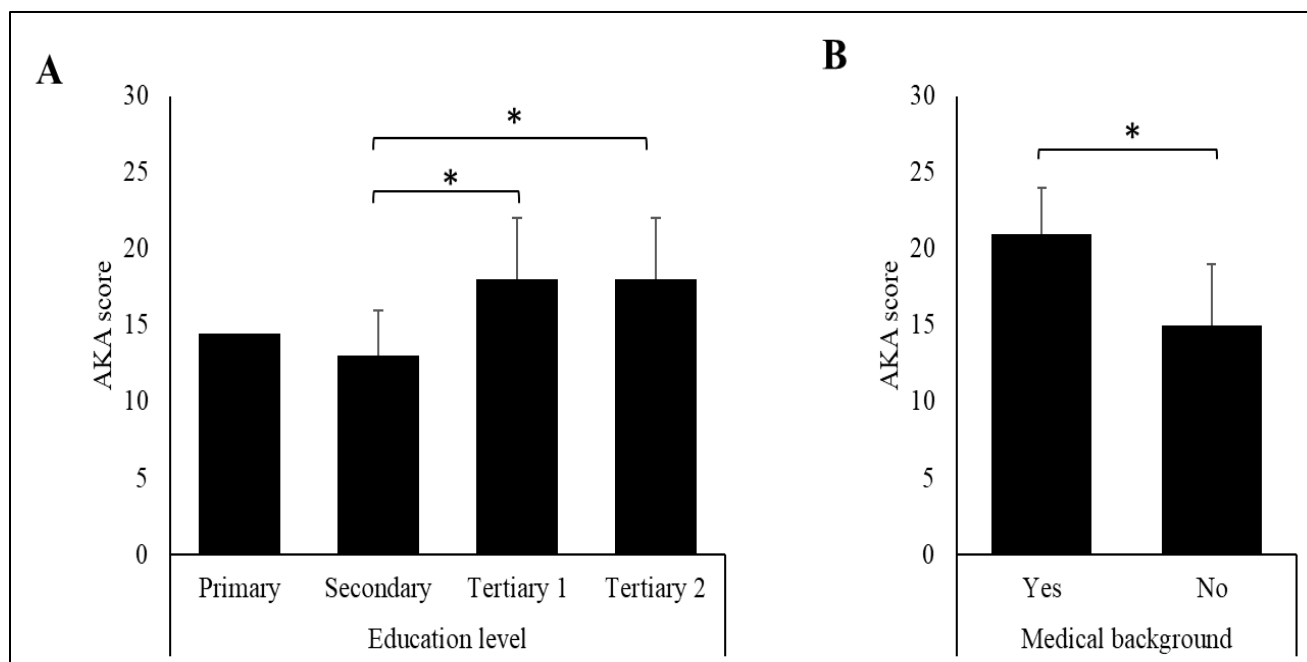


Figure 1. Comparison of Total AKA scores by Education Level and Medical Background. (A) Bar chart (median  $\pm$  IQR) of total AKA score by education level. Groups were compared using the Kruskal-Wallis test with post-hoc Dunn's multiple pairwise comparison test, revealing higher AKA scores in tertiary education groups compared to secondary education. \* indicates  $P < 0.001$ . (B) Bar chart (median  $\pm$  IQR) of total AKA scores by medical background (yes/no). Groups were compared using the Mann-Whitney U test, showing a higher median AKA scores among participants with a medical background. \* indicates  $P < 0.001$ .

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## CASE REPORT

# Audit of Ophthalmological Case Management: A Case Series Review at a Local Health Clinic.

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### Abstract

Eye diseases are among the common cases encountered in health clinics. They range from stable to unstable cases. Nevertheless, there are still unclear approaches taken by health care providers in treating them, especially in relation to urgent referrals or outpatient care requirements. We conducted an audit at a local health clinic involving eye cases encountered in the treatment room, which had been assigned as an emergency during the afternoon session for one week. Of the 68 cases recorded, seven involved eyes. The majority were foreign body cases, which were not referred to ophthalmological care and were not preceded by eye irrigation. Therefore, in-house training on the approach to eye diseases should be conducted from time to time.

**Keywords:** *Eye emergency, eye redness, foreign bodies.*

## Introduction

Symptoms related to eyes encountered in primary care range from red eyes, discharges, and reduced vision to even painful eyes secondary to trauma [1,2]. Given the nature of vision as an important organ function, most patients seek attention as soon as possible at the nearest clinic [3]. Therefore, it is an important role for primary care providers to conduct proper triage in terms of the severity of the injury or complaints. This includes identification of the need for tertiary care or follow-up post-assessment. Eye diseases can present as acute emergencies that require direct attention in the treatment room or emergency room at a health clinic [4]. Nevertheless, even if there is a need for urgent referral from primary care to tertiary care, proper preparation and stabilization of the patient are still needed [5]. This includes the adequate eye examination and necessity of eye irrigation in selected cases as well as monitoring of vital signs [6]. In cases of doubt, even if the patient looks stable, a second opinion should be sought for proper management. Indeed, common rules of the correct approach at the first visit should be maintained.

## Methodology

An audit was conducted in a local health clinic involving cases that required emergency attention in the treatment room and emergency room during the afternoon sessions over a five-day period. Afternoon sessions were selected due to the smaller number of patients during this time, and typically the treatment room focuses more on non-communicable disease follow-up, such as electrocardiography (ECG) procedures and diabetic complications, which could affect the efficiency of managing other emergency cases. This audit aims to identify any mismanagement in treating common eye diseases in primary care. The patient's age, gender, system involvement, diagnosis and management given were recorded. Other diseases not covering eyes were excluded. Those cases that were seen in usual outpatient room were also excluded. This audit has been approved by the Family Medicine Specialist in

charge of the clinic and Kulliyah of Medicine elective posting committee (IIUM305/13/26/1). Seven cases that fulfill the criteria have been selected for the case series discussion.

### Case series seen at the treatment room or emergency unit.

#### Case 1:

A 25-year-old woman presented with unilateral left eye redness, discomfort, and discharge for two days. This case was sent directly to the treatment room to be assessed by a medical officer due to the unilaterality and associated pain, as noted by a medical assistant at triage. Upon assessment, the pain was noted to be mild and more like discomfort. It is not common for conjunctivitis to be unilateral, but it is still possible, as in this case. There was no history of trauma, itchiness, or insect bite. She was not wearing any contact lenses. The need for the treatment room was not indicated and was not an emergency. The patient was discharged with chloramphenicol eye drops without any follow-up.

#### Case 2:

A 29-year-old man presented with right eye discomfort after glue splashed into it one hour prior while working. He had difficulty opening his right eyelid. Upon arrival, he was rushed to the treatment room but promptly referred to a tertiary center due to difficulty in examining the affected eye. His left eye was noted to be normal. His vital signs were stable. No additional treatment was given at the health clinic.

#### Case 3

An 8-year-old girl presented with right eye redness and pain after alleged exposure to soil dust in her right eye two hours prior the clinic visit. The incident happened while she was performing activities on the school field. Her vital signs were stable. Visual acuity could not be performed as the patient was in pain. External eye examination revealed a watery right eye with conjunctival redness. There was no periorbital or eyelid swelling. The pupils' sizes were equal and responsive to light. No relative afferent pupillary defect was detected. Eye irrigation with normal



saline was performed. She was later discharged with Hypromellose 0.3% eye drops.

#### **Case 4**

A 15-year-old girl presented with left eye discomfort after allegedly having grass thrown into her left eye by a grass-cutting machine. The incident happened while she was walking back home from school. She also complained of reduced vision. Visual acuity could not be performed because the patient was not comfortable. External eye examination revealed a watery left eye with conjunctival redness. There was mild left eyelid swelling. The pupils' sizes were equal and responsive to light. No relative afferent pupillary defect was detected. Eye irrigation was performed vigorously. Her symptoms improved, and she was discharged with Hypromellose 0.3% eye drops.

#### **Case 5**

A 12-year-old boy presented with right eye pain and redness after his right eye was accidentally hit by a bat. He had no other complaints. His vital signs were stable. External eye examination revealed conjunctival redness in the right eye with a swollen and inflamed periorbital area. The pupils' sizes were equal and responsive to light. No relative afferent pupillary defect was detected. The ocular movement was intact. He was discharged with chloramphenicol eye drops for one week, with no further follow-up.

#### **Case 6**

A 72-year-old female presented with a sudden onset of isolated redness in her left eye, that had lasted for two days. She had no history of orbital or periorbital swelling, itchiness, discharge, or pain. There was no prior history of trauma or insect bites. She was hypertensive but not diabetic. She was not on anticoagulants and has had no similar episodes before. On examination, her vital signs were stable. External eye examination revealed an isolated subconjunctival hemorrhage at the inferolateral site of the left eye. The pupils were equal in size and responsive to light. No relative afferent pupillary defect was detected. Ocular movement was intact. She was discharged

with oral antihistamine (loratadine) and Hypromellose 0.3% eye drops.

#### **Case 7**

A 39-year-old man presented with bilateral eye pain and redness for three hours following accidental exposure of the eyes to car wash detergent. The incident happened when he was trying to reach the uncapped detergent container on a shelf, and the fluid accidentally fell onto his face. He also complained of reduced vision following the injury. External eye examination revealed bilateral eye redness with clear discharges. The pupils were equal in size and responsive to light. No relative afferent pupillary defect was detected. Ocular movement was intact. He was discharged with chloramphenicol eye drops for one week without further follow-up.

#### **Discussion**

The emergency room at the health clinic is intended to treat life-threatening conditions or any condition that requires urgent treatment, assessment, or stabilization [7]. The decision to triage patients depends on the screening team at the clinic. However, the emergency unit can be burdened by non-indicated cases or incorrect assessments by front-line staff [7,8]. This can lead to inefficiencies, as waiting times may increase due to the high number of non-indicated cases [7,8]. This scenario is reflected in this audit, where unnecessary eye cases, such as conjunctivitis, were treated in the emergency setting. These cases ranged from isolated subconjunctival hemorrhages to trauma and chemical injuries, allowing us to analyze whether the management provided was appropriate or not. This audit also reveals that basic and fundamental eye examinations, which should be initiated at the primary care level, were not conducted appropriately. Visual acuity assessments were absent in all cases highlighted in this report. The possible reasons for this issue include the discomfort patients experience when in eye pain, or that the assessments were performed but not documented by the doctors. A simple handheld

Snellen chart can be used if it is difficult for the patient to undergo a formal assessment at the clinic [4,5]. Bedside ophthalmoscopic eye examinations were also absent in all cases, despite being a simple procedure that can be performed by junior doctors or support staff. It is important to at least check for the presence of a red reflex and the absence of papilloedema [4,5]. These assessments are feasible even when the pupils are not adequately dilated, as most government health clinics do not have pupil-dilating eye drops. Nevertheless, the experience and confidence level of the treating doctors play an important role in the execution of the procedure. This is evident in this case series, as most of the treating doctors are young medical officers with less than two years of experience. The inadequacies of each case are summarized in Table 1.

According to universal guidelines, any case of a foreign body in the eye should be preceded by adequate eye irrigation and an assessment for eye laceration or abrasion [9]. This means the case should be referred for a proper eye examination, including a slit lamp examination [10]. However, in this audit, all cases involving a foreign body (cases no. 3 and 4) were not referred for further assessment or given a follow-up. Some were not even managed with optimal normal saline eye irrigation. This is quite common, as healthcare providers often mistakenly consider these cases low-risk if the foreign body can be removed. However, a laceration can result from a foreign body, and the severity can only be fully assessed by a slit lamp examination, even if the patient does not complain of red eyes or pain [9,10].

Although subconjunctival hemorrhage can be caused by orbital trauma, it is not necessarily an emergency case [11]. History should be explored before sending all cases with presumed subconjunctival hemorrhage directly to the emergency room. Subconjunctival hemorrhage can be idiopathic in the elderly (as in case no. 6), stable, and self-limiting, and it should resolve on its own [12]. Therefore, the use of the emergency room in this case is indeed not justifiable. The

antihistamine prescribed for this patient was also inappropriate. It is usually indicated for those with allergic conjunctivitis or an acute viral upper respiratory infection, presenting with a runny nose or cough. Antihistamine usage is totally unacceptable for isolated subconjunctival hemorrhage.

The utilization of the emergency room for ophthalmology cases at health clinics should be reviewed. Most of the time, stable eye conditions can be evaluated in a consultation room instead of the emergency unit [13]. According to this audit, the four cases that are indeed indicated to be managed in the emergency room are glue splashed in the right eye, an alleged eye organic foreign body (soil and grass), trauma by a bat, and chemical conjunctivitis [13]. Surprisingly, the majority of these cases (cases no. 3-5 and 7) were not referred to the eye team. Chemical conjunctivitis can result in blindness. Even if topical antibiotics have been applied, daily eye examinations are required for close monitoring [14].

Therefore, it is clear that a common safe approach should be applied by primary care providers in dealing with eye diseases. This includes complete and thorough history-taking and systematic eye and other relevant examinations. The eye assessments conducted among all these cases were not comprehensive. The assessment should include: visual acuity using a Snellen chart or similar tool; performing external inspection of the eyes, eyelids, and surrounding structures; performing a pupil examination to check for size, shape, reactivity to light, and accommodation; assessing ocular motility; inspecting the conjunctiva and sclera to look for redness, discharge, jaundice, or other abnormalities; assessing the cornea using a penlight or ophthalmoscope to check for clarity, depth, and any signs of inflammation; and, last but not least, examining the retina, optic disc, and vessels using an ophthalmoscope [15]. The role of the fluorescein eye test should be made compulsory in all eye cases involving an alleged foreign body or trauma in order to identify any possible corneal

abrasion or laceration that would require further ophthalmological intervention [15,16]. We summarize the recommended eye examination and assessment flow in primary care in Figure 1 below [15, 16].

Indications for eye referral should always be observed and include sudden vision loss or significant visual changes, severe eye pain or trauma, suspected retinal detachment, high intraocular pressure or other signs of acute glaucoma, persistent or severe eye infections, or any condition not responding to initial treatment or requiring specialized care [16]. At the point of discharge from primary care, patients should be educated on the importance of seeking early treatment if any new symptoms occur and on scheduling follow-up visits to monitor treatment response and adjust management as necessary. If primary care providers are unsure of the diagnosis or depth of injury in any situation of a stable eye condition, a proper ophthalmological follow-up should be arranged.

### **Conclusion**

Eye triage should be conducted properly, beginning at the primary care level. Even though some stable cases are seen in an emergency setting, management should not be limited to topical antibiotics only. Each eye case should be

considered severe until proven otherwise. Indications for follow-up and referral should be observed in all ophthalmological cases. Safety netting advice should be emphasized to all patients to seek early medical attention if their condition worsens.

### **Acknowledgement**

We would like to express our gratitude to the Family Medicine Specialist in charge of the Primary Health Care clinic and the staff working there.

### **Ethics**

Permission to conduct the audit was obtained from the Family Medicine Specialist in charge of the clinic and Kulliyyah of Medicine elective posting committee (IIUM305/13/26/1).

### **Conflict of interest**

None

### **Authors contribution**

MSE: Idea, manuscript writings and formatting

NAF: Data collection

Table 1. Descriptive Analysis of Case series

No	Age	Gender	Chief complaint	Associated symptoms	Visual Acuity	RAPD	Other eye examinations	Diagnosis	Referral	Management at clinic	Follow up	Experience treating doctor (medical officer)
1	25	Female	Left eye redness	Eye discomfort	X	X	X	Acute conjunctivitis	X	Chloramphenicol eye drop	X	2 years
2	29	Male	Right eye discomfort	Inability to open eyelids	X	X	X	Chemical injury (glue)	/	X	X	3 years
3	8	Female	Right eye pain	Eye redness	X	/	Pupils' size and reflex	Alleged foreign body	X	Eye irrigation with Normal Saline	X	2 years
4	15	Female	Left eye discomfort	Reduced vision	X	/	Pupils' size and reflex	Alleged foreign body	X	Eye irrigation with Normal Saline	X	1 year
5	12	Male	Right eye pain	Eye redness	X	/	Pupils' size and reflex Ocular motility	Trauma	X	Chloramphenicol eye drop	X	1.5 years
6	72	Female	Left eye redness	-	X	/	Pupils' size and reflex Ocular motility	Subconjunctival Hemorrhage	X	Oral antihistamine Hypromellose 0.3% eye drops	X	1.5 years
7	39	Male	Bilateral eye pain	Eye redness, Reduce vision	X	/	Pupils' size and reflex Ocular motility	Chemical injury	X	Chloramphenicol eye drop	X	1.5 years
Overall percentage of eye examination been conducted					Visual acuity	RAPD	Other examination	Referral percentage	Referral	Follow up percentage	Follow up	
					0%	71%	71%		14%		0%	

X – not done; / - done

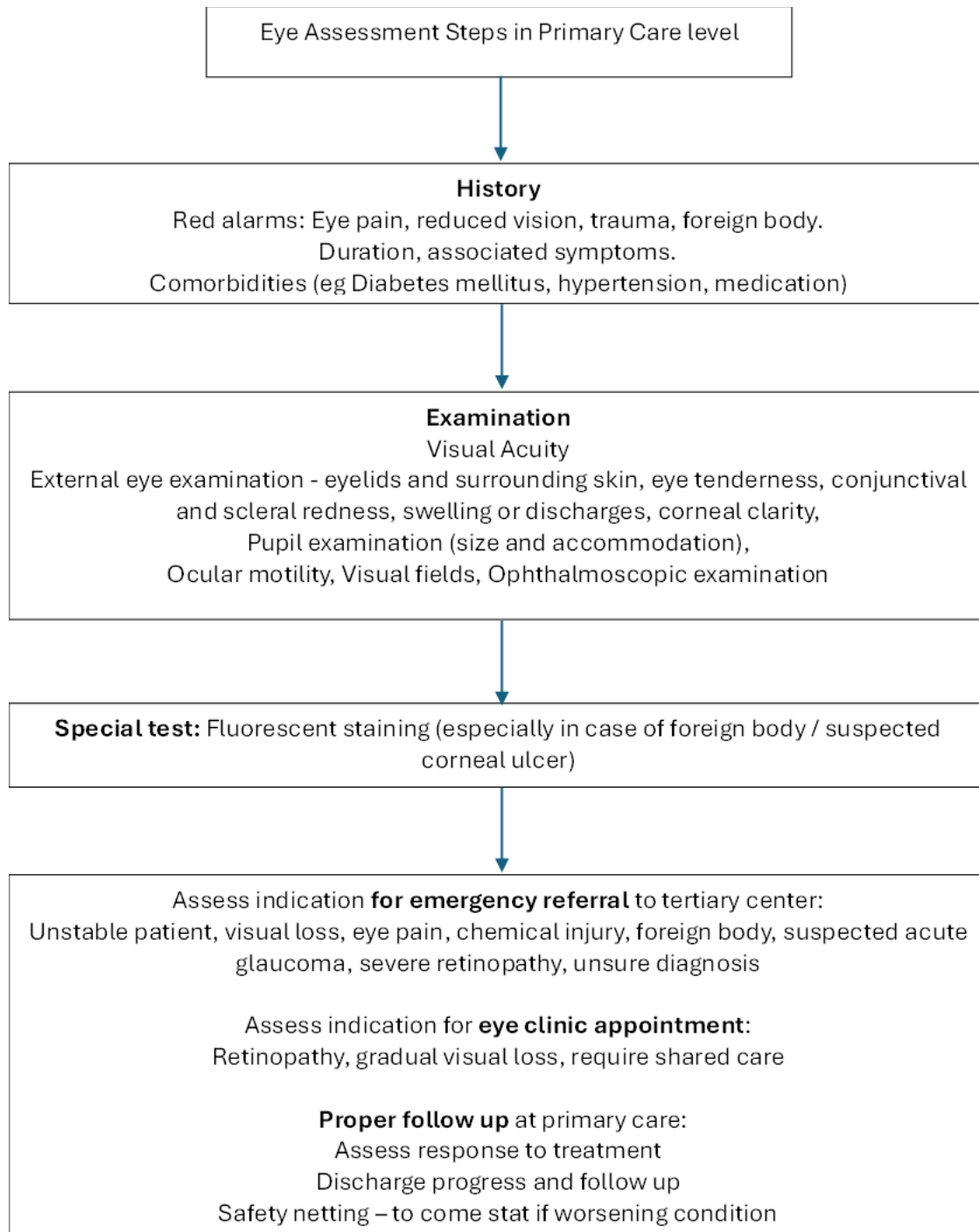


Figure 1. Algorithm for Eye Assessment in Primary Care.

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## CASE REPORT

# Rituximab-induced Acute Thrombocytopenia in a Patient with Mixed Connective Tissue Disease: A Case-based Review.

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### Abstract

Rituximab-induced acute thrombocytopenia (RIAT) is a potential adverse drug reaction associated with the use of this therapeutic agent. Majority of the reported cases involved patients with haematological malignancies and its occurrence in autoimmune connective tissue diseases is rare. The pathogenesis of RIAT remains unclear but immune-mediated pathway has been implicated in the mechanism. RIAT runs a benign course and recovery ensues after its onset. Although life-threatening haemorrhage is uncommon, the risk remains significant with severe thrombocytopenia. Despite being rare, the clinicians should be aware of this phenomenon. Post-administration monitoring of blood counts is pivotal in order to identify its occurrence. This report aims to highlight a case of RIAT with bleeding tendency in a woman with active mixed connective tissue disease.

**Keywords:** *Bleeding, mixed connective tissue disease, rituximab-induced acute thrombocytopenia.*

## Introduction

Rituximab, a chimeric anti-CD20 monoclonal antibody, has an established role in the treatment of lymphoid malignancies and certain autoimmune diseases. Peripheral blood cytopenia, both late-onset and acute-onset, is a recognized side effect associated with the use of rituximab [1]. Late-onset cytopenia (pancytopenia, isolated neutropenia, thrombocytopenia or anaemia) develops several weeks to months after the administration of rituximab and is relatively common with Cattaneo et al reporting an overall frequency of 29.8% [2]. On the contrary, acute cytopenia occurring within a few hours to a few days following the administration of rituximab is rare and of which, thrombocytopenia is the commonest abnormality reported [3,4]. It is evident that patients with haematological malignancies had a higher risk to develop rituximab-induced thrombocytopenia compared to patients with autoimmune diseases [5]. Rituximab has been shown to be an effective treatment option for autoimmune inflammatory rheumatic diseases which are resistant to conventional immunosuppressants [6,7]. With the increasing usage of rituximab, more cases of rituximab-induced acute thrombocytopenia (RIAT) in patients with autoimmune connective tissue diseases are anticipated despite RIAT being a rare adverse drug reaction.

## Case report

A 48-year-old woman presented with progressively worsening arthritis involving both knees and shoulders. She had mixed connective tissue disease (MCTD) with predominant systemic lupus erythematosus (SLE) features for 14 years. Her disease had become increasingly refractory to conventional disease-modifying antirheumatic drugs (DMARDs) and she experienced recurrent debilitating arthritis in recent months. Her latest serological profile showed raised titre of antinuclear factor (ANF 1:320 cytoplasmic, speckled), strongly positive anti-Jo-1, anti-nRNP, and anti-Ro52, positive

anti-Sm, anti SSA/Ro and anti-double-stranded DNA (64 IU/L).

Upon review, active synovitis was noted at her shoulder and knee joints. There was no fever, joint deformity or mucocutaneous lesions. Examination of other organ systems was unremarkable. Investigations revealed elevated erythrocyte sedimentation rate (ESR 49 mm/Hr) and C-reactive protein (CRP 47.7mg/L). Her full blood counts (FBC), liver function test (LFT), renal profile (RP), and urine microscopy were normal. Determined to be having active MCTD, she was initiated on intravenous (IV) parecoxib, IV methylprednisolone, hydroxychloroquine (HCQ) and mycophenolate mofetil (MMF) alongside intra-articular triamcinolone acetone for the knees. The methotrexate (MTX) was continued. Her knee arthritis improved but the left shoulder remained in pain, albeit of lesser degree. The plan to step up therapy to rituximab was discussed prior to discharge.

She was readmitted two weeks later with persistent left shoulder inflammation despite on prednisolone, HCQ, MTX, MMF, and analgesics. She consented to treatment with rituximab. Her baseline investigations prior to receiving rituximab revealed a total white cell count (TWBC) of  $11.6 \times 10^9/L$ , haemoglobin (Hb) of 13.7 g/dL, and platelet (PLT) count of  $139 \times 10^9/L$ . Following the premedication of paracetamol, IV chlorphenamine, and IV methylprednisolone, IV rituximab 1000mg was given. The infusion process was uneventful. The patient requested to be discharged after the treatment as she claimed to feel better.

However, she was readmitted after a day when she developed spontaneous bruises on her body. On examination, multiple purpuric and ecchymotic lesions were noted on her limbs and abdominal wall alongside subconjunctival haemorrhage at the right eye (Figure 1). She was haemodynamically stable and there were no clinical signs of gastrointestinal, urogenital, or



intracranial bleed. Immediate repeat PLT count was  $40 \times 10^9/L$  which further dropped to  $3 \times 10^9/L$  within 12 hours. The TWBC was  $13 \times 10^9/L$  and Hb was 15.1 g/dL. Coagulation profile was normal with international normalized ratio at 0.96 and activated partial thromboplastin time at 36 seconds. Considering the temporal relationship between the rituximab infusion and the drastic decrease in PLT count, the diagnosis of rituximab-induced acute thrombocytopenia (RIAT) was made. She was transferred to intensive care unit (ICU) for close monitoring. A three-day course of intravenous methylprednisolone one gram daily was initiated. All other immunosuppressant agents were withheld. Eight units of platelet concentrates were transfused in a span of two days. Further evaluation showed elevated CRP at 114.5 mg/L, a negative Coombs test, normal complement C3 and C4 level (1.0 g/L and 0.21 g/L respectively), normal RP and LFT. Peripheral blood film revealed thrombocytopenia but no platelet clumps or abnormal cells seen. She remained stable in ICU. The post-transfusion PLT count was  $23 \times 10^9/L$ .

On day 3 after the onset of RIAT, she decided to self-discharge against medical advice and sought treatment in other healthcare facilities for monitoring of the blood counts at her own discretion and risk. The course of the intravenous methylprednisolone was interrupted with the third dose administered on day 4 after the onset of RIAT, along with platelet transfusion when she presented again with thrombocytopenia (PLT count of  $11 \times 10^9/L$ ). Despite her frequent movement, she did not develop any further bleeding complications. Her subsequent PLT counts gradually improved. During her follow-up review 12 days after the onset of RIAT, all the haemorrhagic manifestations had resolved. Her PLT count had returned to baseline level ( $134 \times 10^9/L$ ). Anticipating the possible recurrence of RIAT with the risk of life-threatening haemorrhage, the clinical decision was to discontinue the subsequent dose of rituximab.

## Discussion

Rituximab-induced acute thrombocytopenia (RIAT) is rare in autoimmune connective tissue diseases (CTD) [8,9,10,11]. Majority of the cases were reported in patients with haematological malignancies (Table 1). The pathogenesis of RIAT has not been fully elucidated. Kong et al identified the presence of rituximab-dependent anti-platelet antibodies in three lymphoma patients who developed RIAT following the first exposure to the drug, suggesting an immune-mediated pathogenetic pathway [12]. Other proposed mechanisms of RIAT observed in patients with haematological malignancies include cytokine release syndrome with endothelial damage followed by platelet aggregation resulting in thrombocytopenia, binding of circulatory CD20 antigen to platelet causing immune-mediated cell destruction, and disseminated intravascular coagulopathy-like reaction causing platelet consumption [13,14,15]. Massive tumour burden, bone marrow invasion, splenomegaly, a relatively low platelet count prior to the administration of rituximab, and a high platelet distribution width have been linked to a higher risk of developing thrombocytopenia in patients with haematological malignancies while advanced age and chronic kidney disease are significant risk factors in patients with autoimmune bullous diseases [5,13,14,16]. As for autoimmune CTD, the evidence is still lacking and more robust clinical trials are needed to establish the pathogenesis and to determine the risk factors of RIAT in these patients.

RIAT has been reported when rituximab is used in various settings. It can arise when rituximab is given as a monotherapy or in combination with a chemotherapy regimen [3,4,8,9,11,13]. It may occur following the first dose of rituximab as in this case or only appear after repeated exposure to the drug [8,9,11,13,14,17]. In RIAT, the thrombocytopenia develops early, usually in a few hours after the administration of rituximab, and typically reaches a nadir within a day [3,4,18,19,20]. Its abrupt onset can be missed if

the blood counts are not monitored particularly when rituximab is administered in an out-patient setting, hence possibly leading to underdiagnosis of this event. At present, there remains no clear recommendation for the frequency of blood count monitoring during the immediate post-transfusion period when rituximab is utilized in rheumatic diseases [21]. While the platelet count in RIAT can decline rapidly to a critical level, haemorrhagic manifestations are not common [3,4,13,14,17,18,19,20,22]. Nonetheless, the risk of major bleeding remains high when the platelet count is less than  $20 \times 10^9/L$  [23]. Hence, platelet transfusion is often given as a precautionary measure in severe thrombocytopenia. Interestingly, when the cases of RIAT in autoimmune inflammatory rheumatic diseases were analysed, haemorrhagic manifestations seemed to be more frequently reported (mucosal bleed, haematuria, bloody stool) [8,9,11]. This patient had also developed mucocutaneous bleeding along with the thrombocytopenia. This observation may suggest that even though RIAT is uncommon in autoimmune CTDs, there may be a higher tendency for patients to develop bleeding complications when it does occur. Besides the low platelet counts, other determinants of the bleeding risk need to be explored, particularly those contributing to platelet dysfunction [24].

RIAT is a transient event and resolution within one to two weeks with gradual recovery of platelet counts is expected in most cases [3,8,11,13,17,20,22]. The principal management strategies include close observation for any bleeding tendencies, intensive monitoring of the blood counts, and platelet transfusion when indication arises. The usage of corticosteroids and intravenous immunoglobulins has been implicated in the treatment of RIAT, by promoting the recovery of the platelet counts

[8,25]. Some authors reported the recurrence of RIAT with reinitiation of rituximab [8,13,18,19]. In other cases, the patients continued to receive the subsequent cycles of chemotherapy regimen with rituximab safely without any recurrence [3,17]. At present, there is no plausible explanation to this peculiar nature of RIAT.

## **Conclusion**

Despite RIAT being a rare phenomenon, the emerging reports of this condition in autoimmune connective tissue diseases should raise awareness among clinicians regarding this adverse effect of rituximab. Close monitoring of blood counts after the administration of rituximab is crucial in order to capture its occurrence and minimize the possibility of catastrophic haemorrhage. Future development of expert consensus on the management of RIAT is highly anticipated.

## **Conflict of Interest and financial disclosures**

None

## **Informed Consent**

Written informed consent was obtained from the patient for the publication of this report and the accompanying images.

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We would like to express our gratitude to all the healthcare providers involved in the care of this patient.

## **Authors contribution:**

LHS: Manuscript writing and formatting

WS: Ideas, case management, data collection, and review of the manuscript

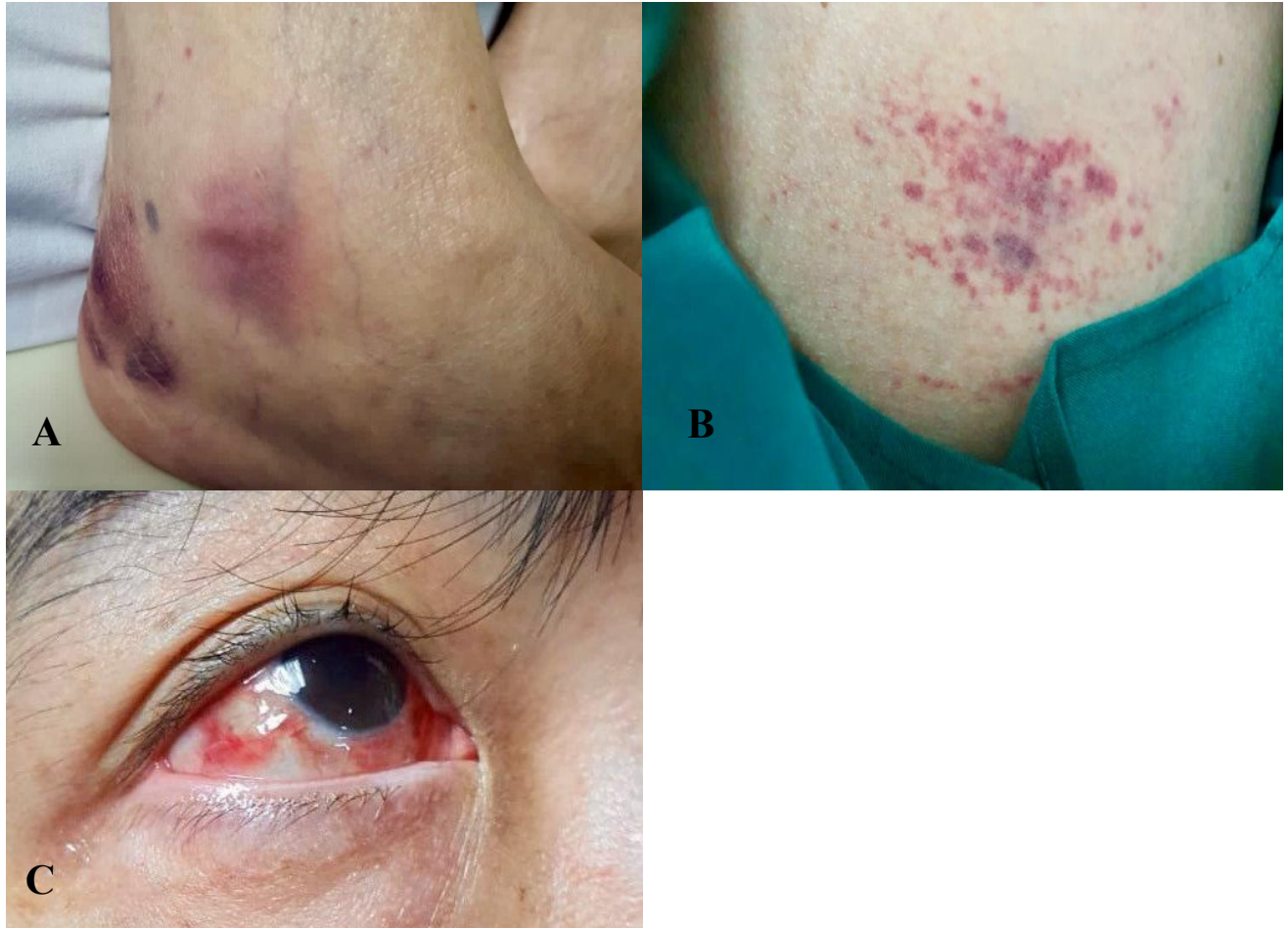


Figure 1. A. Ecchymotic patches on the right ankle. B. Purpuric spots on the abdominal wall. C. Subconjunctival haemorrhage at the right eye.

Table 1. Summary of case reports of rituximab-induced acute thrombocytopenia (RIAT)

Study	Age (Y) /Sex	Indication of rituximab	Occurrence with first use	Treatment regimen	Time interval*	PLT nadir (X10 <sup>9</sup> /L)	Bleeding / PLT transfusion	PLT recovery	Recurrence with subsequent use
<i>El-Osta et al</i> [3]	66; F	MCL	Yes	Combination	Within hours	16	No / No	6 days	No
<i>Ram et al</i> [4]	71; F	MCL	No	Combination	8 hours	10	No / No	5 days	Not known
<i>Yudhishdran et al</i> [8]	36; F	SLE	Yes	Single agent	10 days	5	Yes / Yes	10 days	Yes
<i>Akpabio et al</i> [9]	39; F	SLE	Yes	Single agent	12 days	59	Yes / No	7 days	Not known
<i>Shah et al</i> [10]	35; F	SLE	Yes	Single agent	3 days	41	No / No	10 days	Yes
<i>Endo et al</i> [11]	72; F	GPA	No	Single agent	3 days	7	Yes / Yes	2 weeks	Not known
<i>Omura et al</i> [13]	74; M	FL	No	Combination	1 day	14	No / Yes	Within 1 week	Yes
<i>Jiang et al</i> [14]	56; F	FL	No	Combination	1 day	26	No / No	Within 1 week	Yes
	63; F	SMZL	Yes	Combination	1 day	25	No / No	Within 1 week	Yes
<i>Sadashiv et al</i> [17]	63; F	MCL	Yes	Combination	1 day	5	No / Yes	4 days	No
	72; M	MCL	Yes	Combination	1 day	10	No / Yes	5 days	No
	60; M	MCL	No	Combination	3 days	11	No / Yes	4 days	No
	64; F	MCL	Yes	Combination	1 day	3	No / Yes	13 days	No
	76; M	MCL	Yes	Combination	1 day	26	No / No	4 days	Not known
<i>Yi et al</i> [18]	58; M	MCL	Yes	Combination	1 day	24	No / No	3 weeks	Yes
<i>Rosado et al</i> [19]	63; M	MCL	No	Combination	1 day	15	No / Yes	3 days	Yes
<i>Ureshino et al</i> [20]	65; M	FL	Yes	Combination	1 day	5	No / Yes	Within 1 week	Not known
<i>Otrock et al</i> [22]	41; M	HCL	Yes	Combination	1 day	7	No / Yes	1 week	Not known
	64; M	MCL	Yes	Single agent	1 day	10	No / Yes	A few days	Not known
This study	48; F	MCTD	Yes	Single agent	1 day	3	Yes / Yes	12 days	Not known

M: male, F: female, Y: years, PLT: platelet, MCL: mantle cell lymphoma, SLE: systemic lupus erythematosus, GPA: granulomatosis with polyangiitis, FL: follicular lymphoma, SMZL: splenic marginal zone lymphoma, HCL: hairy cell leukaemia, MCTD: mixed connective tissue disease

\*Time interval to documentation of thrombocytopenia after rituximab administration

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## CASE REPORT

# Evaluating the Impact of Targeted Shoulder Exercises on Shoulder Impingement Syndrome: A Comprehensive Case Study.

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### Abstract

Shoulder impingement syndrome (SIS) is a common cause of shoulder pain, especially among the elderly, particularly in women. SIS significantly affects shoulder function in daily activities and work quality. This case study focused on a 57-year-old female who experienced moderate to high-intensity shoulder pain and limited range of motion of left shoulder joint after falling at home. The objective was to alleviate pain and improve shoulder function through shoulder exercises. Mobility exercises for the shoulder joint and strengthening exercises for the serratus anterior, rhomboid and trapezius muscle were administered, resulting in reduced pain and increased range of motion. The study demonstrated that shoulder exercises had a beneficial effect on patient with SIS. Addressing the specific muscle weaknesses is crucial to enhancing overall shoulder function. This research highlights the importance of strengthening exercises for shoulder muscles as management strategies in SIS.

**Keywords:** *Impingement syndrome, physiotherapy, shoulder exercises, shoulder pain.*

## Introduction

Shoulder impingement syndrome (SIS) is a prevalent disability, affecting approximately 74% of patients with shoulder pain [1]. Women are particularly prone to experiencing shoulder pain more frequently than men due to their smaller shoulder frames, lower muscle strength in the shoulder region, and lower aerobic capacity. Joint laxity appears to be influenced by sex hormones, accompanied by ageing and degenerating joint; therefore, women are more susceptible to musculoskeletal disorders, including shoulder pain [2].

Patients with SIS suffer from painful elevation and depression of the arm when performing any overhead motion between 60° and 120°, and they experience pain when lying on the affected side [3,4]. SIS manifests in two distinct pathologies: subacromial impingement and internal impingement. Subacromial impingement can be further categorized into two types, occurring anterolaterally at the anterior acromion and the coracoacromial ligament [3,4]. In contrast, internal impingement involves the greater tuberosity of the humerus and the articular surface of the rotator cuff, which impinge on the posterosuperior glenoid when the shoulder is externally rotated and abducted [1].

A simple fall can cause an acute rotator cuff tear and a fall-related tear. A fall on the outstretched arm can result in adduction and internal rotation of the shoulder joint, leading to stress on the supraspinatus-infraspinatus tendons [5]. Repetitive rubbing of the injured rotator cuff tendon between the humerus and the outer edge and lower edge of the acromion leads to more swelling and further narrowing of the space, resulting in pain and irritation [5,6]. SIS can affect patients of any age; however, the pre-existing factor such as degenerative changes, overuse muscle, or reduce tensile strength can have devastating effects to the shoulder joint [5]. Positional faults such as slouching can also lead to SIS. The drooping shoulder that results from weakness of the rotator cuff and scapular muscles can cause the translation of the humeral head and scapula. Consequently, there is a narrowing of the

subacromial spaces, leading to pain during any overhead motion [1,6].

There is a wide range of treatments available for SIS, including both surgical and conservative options. However, physiotherapy alone can provide favourable outcomes comparable to surgery, without the costs and complications associated with surgical intervention [1,6]. Physiotherapy treatments for SIS often include exercises, manual therapy, such as joint mobilization and soft tissue manipulation, and modalities, for example, hot pack, transcutaneous electric nerve stimulation (TENS), ultrasound, and infrared therapy [7,8].

Manual therapy techniques and modalities are often recognized as a “passive” treatment that provide quick relief to the patients. However, this can lead patients to become overly reliant on their physiotherapist and engage less in exercises [9]. Therefore, the objective of this case study is to determine whether exercises can provide pain relief and increase the range of motion without using any manual therapy and modalities for patients with SIS.

## Case description

### *Subjective examination*

On April 10, 2023, a 57-year-old female patient presented herself at the physiotherapy department, reporting discomfort in her left shoulder resulting from a fall she experienced one month prior. Following the incident, she did not seek medical attention. However, as the pain increased and her ability to use her left shoulder became impaired, she visited her general practitioner and was diagnosed with SIS. The physician prescribed a painkiller and referred her for physiotherapy. Before the onset of shoulder pain, the patient actively fulfilled her role as a housewife and participated in tai chi exercises during her leisure time. Unfortunately, since experiencing shoulder pain, she has been unable to perform household chores and discontinued tai chi practice.

During her first visit, she complained of aching pain in the left shoulder joint (referred to as P1)



with a numeric pain rating scale (NPRS) score of 7 out of 10 when completing any overhead motion such as tying her hair, engaging in prolonged hand activity such as driving, and sleeping on the left side. However, after taking the prescribed painkiller, the pain subsided to a score of 2 out of 10. She also complained of aching pain in the left scapula region (referred to as P2), with NPRS score of 4 out of 10, particularly when engaging prolonged sitting or carrying out heavy lifting during house chores. The pain would immediately reduce to a score of 2 out of 10 after lying down. Besides the prescribed painkiller, she claimed she did not consume any medication or supplement, as she was healthy and had no other diseases. Figure 1 provides a detailed overview of the pain assessment findings.

### ***Physical examination***

The patient presented with a normal gait upon entering the physiotherapy department; however, a reduction in arm swing was observed on the left side. During the postural examination, it was found that the left shoulder appeared drooped, the left scapular was anteriorly tilted and protracted, and there was visible muscle atrophy, particularly in the supraspinatus, infraspinatus, biceps, and deltoid muscles, was evident. On physical examination, she had tenderness grade 2 just below the acromion process and around the glenohumeral joint until the left armpit. Both active and passive range of motion in her left shoulder were limited (Table 1), while active full range of motion (AFROM) and passive full range of motion (PFROM) were observed in her right shoulder joint. Specific details regarding the outcome measures of range of motion of left shoulder that had been measured using goniometer are shown in Table 1.

There was evident overall muscle weakness of the left shoulder, i.e., the shoulder flexor, abductor, external rotator, internal rotator, and extensor. Notably, all cervical spine movements did not elicit pain in the neck or shoulder region.

Next, scapular mobility was assessed. It was observed that the left scapular was tilted anteriorly and protracted at rest, resulting in reduced mobility during shoulder movement. During passive examination of left scapulothoracic articulation, there was a reduction in passive mobility, especially during inferior, medial, and downward rotational glide. The muscle surrounding the left scapular region namely the serratus anterior, rhomboids, lower and middle trapezius, exhibit overall weakness with a Medical Research Council (MRC) muscle strength score of 3 out of 5 compared to the right side, 5 out of 5.

On reviewing the X-ray report, no degenerative changes or fractures were identified on the affected shoulder. Therefore, special tests were conducted to confirm the diagnosis. Drop arm test was negative. Empty can test, Neer's test, and Hawkin-Kennedy test yielded positive results.

The drop arm test and empty can test are diagnostic manoeuvres to identify a possible rotator cuff pathology, particularly tears in the supraspinatus muscle-tendon complex. Neer's test and Hawkin-Kennedy test are clinical manoeuvres to provoke symptoms and identify the potential impingement structures within the shoulder joint [10]. In summary, the findings from the tests aligned with the diagnosis, confirming that the patient was experiencing pathology in the supraspinatus tendon, specifically impingement syndrome.

### ***Treatment and Evaluation***

The case underwent a three-week period and the treatment commenced based on the assessment results. The treatment details for the initial week are presented in Table 2, while Table 3 presents the evaluation of the first treatment.

Table 3 illustrates a notable improvement in the range of motion (ROM) across all movements of the left shoulder joint following the implementation of strengthening exercises targeting the serratus anterior, rhomboid, and trapezius muscles. The AFROM for shoulder

flexion and abduction is established at 180°. Specifically, shoulder flexion exhibited a significant enhancement, with a 16% increase from 90° (50% of the ROM) to 120° (66% of the ROM). Abduction also displayed a meaningful improvement, with an 8% increase, rising from 80° (44% of the ROM) to 95° (52% of the ROM). Notably, internal and external rotations demonstrated more pronounced improvements compared to flexion and abduction. With an AFROM of 90°, internal rotation improved by 44%, while external rotation increased by 33%. These findings underscore the effectiveness of the targeted muscle-strengthening exercises in enhancing shoulder joint mobility.

Hence, a similar protocol was given for the subsequent week with higher intensity. Closed kinetic strengthening exercises were prescribed to enhance the stability of shoulder joint and eventually strengthen the scapular muscles. Table 4 provides an overview of the treatment specifics for the subsequent two weeks, while Table 5 and Table 6 encompass the overall evaluation.

Based on the above findings, NPRS score significantly reduced within three weeks exercise program, with high intensity observed in the first week and a dramatic reduction to zero by the third week. The patient has not taken any painkillers since the second week of treatment. Similar to the range of motion of the left shoulder joint, there was significant improvement, particularly in internal rotation and external rotation movements, which were initially restricted due to pain in the first week. As the pain subsided, the range of motion improved almost to a full capacity by the third week. Therefore, it is evident that exercises can help patients with SIS reduce pain and improve shoulder range of motion. The exercise design is very important to figure out which exercise is most suitable for reducing pain, hence, helping to increase range of motion for the patient. Careful consideration and implementation of an exercise regime tailored to the specific needs and capabilities of the individual are paramount to achieving a successful outcome.

## Discussion

An observation assessment in this study revealed that the patient exhibited a drooping shoulder, as well as an anteriorly tilted and protracted scapular. These postural faults indicate weakness in the scapular musculature [4]. That statement aligns with MRC muscle strength score assessment of the patient, which revealed weakness in the scapular musculature, specifically the serratus anterior, rhomboid, lower trapezius, and middle trapezius muscles. This finding is considered important because the scapular musculature serves to stabilize and rotate the scapular during movement, thereby weakening or dysfunction can cause abnormal translation of the scapular at rest and during motion, resulting in SIS [11]. In addition, degenerative changes and postural faults can cause translation of the humeral head, leading to the narrowing of the subacromial spaces and eventually resulting in tendon impingement [1,5,12]

The scapula stability and mobility are maintained by the coupled action of serratus anterior and lower trapezius muscles [8,11,13]. Therefore, push-up exercises have been included in the patient's exercise regime to strengthen the serratus anterior muscle. The push-up was the primary treatment to alleviate pain and repositioning of the scapula because it activates the serratus anterior muscle and minimizes upper trapezius contraction [13].

The other stabilizing muscles such as rhomboid, middle, and lower trapezius are often neglected, while the prime mover muscles, such as upper trapezius, pectoralis major, and deltoid muscles are often targeted in the exercise regime of any shoulder disorder treatment, including SIS [11]. However, the stabilizing muscles are fairly important in the exercise regime because they play an important role in coordinating and maintaining the movement of the shoulder complex [14]. Therefore, isometric exercises and strengthening exercises using resistance band for stabilizing muscles were included in the exercise regime. These muscles should be strong enough

to correct the positional faults of the patients with SIS, and hence ease the movement of the shoulder joint [11,15].

A closed kinetic chain exercises for serratus anterior, rhomboid and medial trapezius muscles is included in the exercise regime because neuromuscular training is one of the most important components for injury prevention. Exercise in a closed kinetic chain contributes to the inclusion of all muscles, not just the arm but also the muscles of the trunk and legs [16]. In addition, closed kinetic chain exercises generate greater activation of serratus anterior, and middle and lower trapezius muscles compared to the upper trapezius muscle; hence, it may benefit shoulder joint stability [13,15]. Exercise performed in a closed kinetic chain also promotes activation of the antagonist muscles, thereby providing stability of the coupled-action muscles [16].

Besides, research has shown that a closed kinetic chain stimulates mechanoreceptors and contributes to shoulder joint stabilization. As a result, it will facilitate neuromuscular adaptation in response to strength training [15,16]. In contrast to an open kinetic chain, it activates the upper trapezius muscle and puts more stress on the shoulder joint, especially in the 'high five' position possibly leading to re-injury of the shoulder joint [13]. Therefore, considering the patient's condition, a closed kinetic chain is more relevant to the exercise regime.

Systematic reviews have also suggested that strengthening exercises targeting scapulothoracic complex effectively reduce pain and disability in patients with SIS [17,18]. Considering these findings, it is notable that exercises for the shoulder joint and muscles lead to decreases in pain, increases range of motion and improve the functional capacity in patients with SIS.

This case study has some limitation as one could not determine how well patient was doing her home program but had to depend solely on the report given by patient.

## **Conclusion**

Based on the aforementioned findings, it can be concluded that exercises have the potential to effectively alleviate pain and enhance the range of motion of the shoulder joint in individuals with SIS. The design and selection of appropriate exercises play a vital role in determining the effectiveness of pain reduction and improvement in range of motion.

However, it is important to note that the conclusions drawn from this case study should be interpreted with caution due to limited scope and the specific characteristics of the individual involved. The uniqueness of each patient's condition, including their specific symptoms, medical history, and response to treatment, suggests that the outcomes observed in this case may not be universally applicable. To establish more robust and reliable results, it is recommended that future research endeavours incorporate a larger sample size and employ a diverse study design. By including a more diverse range of participants and various methodologies, these future studies can provide a promising understanding of the effectiveness of exercise interventions for individuals with SIS.

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## **Conflict of interest statement**

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare absence of conflicting interests with the funders.

## **Authors contribution**

Both authors contributed equally to the drafting of the manuscript, data collection, analysis and editing the manuscript.

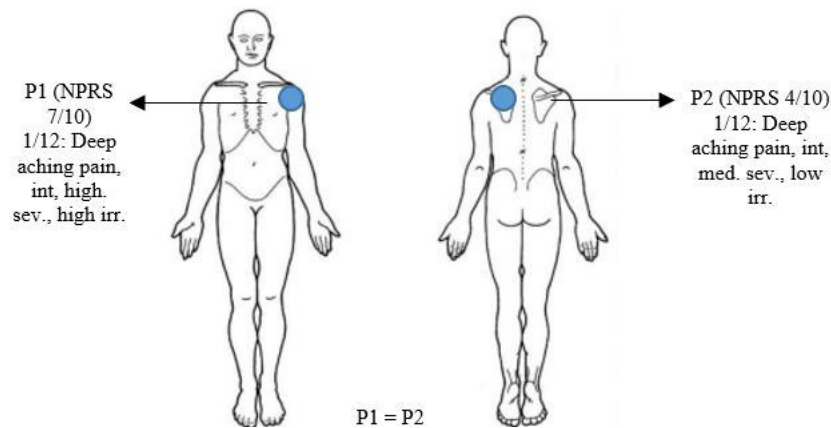


Figure 1. Body chart

Table 1. Range of motion of left shoulder joint.

Movement	Active	Passive	End feel
Flexion	0 - 90°	0 - 120°	Empty
Abduction	0 - 80°	0 - 90°	Springy
Extension	0 - 35°	0- 40° (PFROM)	Firm
Internal rotation	0°	-	-
External rotation	0°	-	-

Table 2. Treatment for patient with SIS at week1.

Purposes	Treatment
To increase scapular musculature strength	1. Serratus anterior strengthening exercises Position: supine lying F: 3x/day; I: 10 sec hold, 10 times, 3 sets; T: Free weight strengthening exercise
	2. Rhomboid & middle trapezius exercises Position: Sitting F: 3x/day; I: 10 sec hold, 10 times, 3 sets; T: Isometric exercise
	3. Lower trapezius exercises Position: Sitting; F: 3x/day; I: 10 sec hold, 10 times, 3 sets; T: Resistant band strengthening exercise
To maintain shoulder mobility	1. Pendulum exercises Position: Standing; F: 3x/day; I: 10 times, 3 sets; T: Mobility exercise

\* F: frequency of exercises; I: intensity of exercise; T: type of exercise

Table 3. Range of motion of the shoulder joint pre and post treatment.

<b>Movement</b>	<b>Pre</b>	<b>Post</b>	<b>Differences</b>	<b>% of improvement</b>
Flexion	0 - 90°	0 - 120°	30°	16%
Abduction	0 - 80°	0 - 95°	15°	8%
Internal rotation	0	0 - 40°	40°	44%
External rotation	0	0 - 30°	30°	33%

Table 4. Treatment for patient with SIS at week 2 and 3.

<b>Purposes</b>	<b>Treatment</b>
To increase scapular musculature strength	<p>1. Serratus anterior strengthening exercises – against the wall exercises Position: standing; F: 3x/day; I: 10 times, 3 sets; T: Closed kinetic chain strengthening exercises</p> <p>2. Rhomboid &amp; middle trapezius exercises – wall push up Position: Standing; F: 3x/day; I: 10 times, 3 sets; T: Closed kinetic chain strengthening exercises</p> <p>3. Lower trapezius exercises Position: Sitting; F: 3x/day; I: 10 sec hold, 10 times, 3 sets; T: Resistant band strengthening exercise</p>
To improve shoulder flexibility	<p>1. Stretching exercises – using stick Position: Standing; F: 3x/day; I: 15 sec hold, 5 times; T: Assisted stretching exercises</p>

\* F: frequency of exercises; I: intensity of exercise; T: type of exercise

Table 5. NPRS score of shoulder joint from week 1 to week 3.

<b>Week</b>	<b>1</b>	<b>2</b>	<b>3</b>
NPRS score	7	2	0

\*NPRS: Numeric pain rating scale

Table 6. Range of motion of shoulder joint from week 1 to week 3.

<b>Movement</b>	<b>Week 1</b>	<b>Week 2</b>	<b>Week 3</b>
Flexion	0 - 120°	0 - 145°	0 - 160° (AFROM)
Abduction	0 - 95°	0 - 120°	0 - 140°
Extension	AFROM	AFROM	AFROM
Internal rotation	0	0 - 30°	0 - 60°
External rotation	0	0 - 40°	0 - 70°

\*AFROM: Active full range of motion

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## CASE REPORT

# Unraveling the Enigma: A Case Series of Human Pentastomiasis Encounters.

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### Abstract

Pentastomiasis is a zoonotic parasitic disease caused by pentastome parasites, typically reported in Africa, the Middle East, and Southeast Asia. The definitive hosts of *Armillifer* spp. (a subclass of pentastomes) are snakes, lizards, and other reptiles. Humans acquire the infestation incidentally by consuming uncooked, infected snake meat or drinking water contaminated with pentastome ova. We report two cases of human pentastomiasis, an uncommon condition today. This case report aims to provide the latest updates on human pentastomiasis, particularly in Malaysia, and promote awareness among primary healthcare providers.

**Keywords:** *Parasitic infection, pentastomiasis.*



## Introduction

Pentastomiasis, also known as "tongue worm" infestation or porocephalosis, was first documented a human case in Egypt in 1847. Since then, the disease has been reported sporadically in Southeast Asia, the Middle East, and Africa [1], predominantly caused by nymphs from the genera *Armilifer* and *Linguatula*. *Linguatula serrata* and *Armilifer armillatus* are the two main parasites, contributing to more than 90% of human infections [2]. Human pentastomiasis is a parasitic disease of zoonotic origin, wherein humans unintentionally become hosts for the parasite. Humans serve as dead-end hosts for pentastomes, acquiring the infection by consuming raw or undercooked snake meat containing the parasite larvae or ingesting water contaminated with pentastome eggs. Therefore, it is usually an incidental finding for unrelated pathology during surgical procedures, postmortem examinations, or radiological investigations [3]. However, the patient can present with clinical signs and symptoms, including fever, nausea, vomiting, diarrhea, jaundice, and abdominal pain. Human pentastomiasis was reported among locals in Malaysia in the 1960s, the last case was reported by Baha Latiff in 2011 [4].

### Case presentation 1

A 58-year-old Aboriginal male presented to the emergency department due to fever and on-and-off abdominal pain for one week. He had no known medical illness previously.

Examination revealed that he was mildly dehydrated but neither pale nor jaundiced. The vital signs were stable, with no documented spike in temperature. His abdomen was soft, mildly tender at the right upper quadrant, with no palpable masses. Digital rectal examination was unremarkable. His laboratory results showed eosinophilia with normal liver enzymes.

A plain abdominal radiograph, performed in supine position, showed multiple C-shaped (crescentic) opacities in all quadrants of the abdomen. A CT scan of the abdomen

complemented the findings of the abdominal radiograph. Since the objective was to locate the calcifications, only plain CT scan without contrast was performed. The CT scan showed multiple crescentic calcifications throughout the body, particularly in the solid viscera and peritoneal cavity. The calcifications were primarily concentrated in the lungs, liver, and mesenteric fat. The calcifications were relatively uniform, measuring 3mm to 6mm. There was no significant lymph node enlargement or bony lesions. The liver was not enlarged with a smooth margin [Figure 1].

In light of the radiological findings, further history was obtained. The patient confirmed that snakes had been a regular part of his diet. He would kill them and eat them raw or cut them into sizeable pieces, then boil the pieces in hot water before slicing them, adding spices, and cooking over an open fire. He started consuming snakes as a child.

Correlating the radiologic findings with the clinical history led to a diagnosis of pentastomiasis. The patient was admitted for one day for observation and then discharged with oral albendazole 400mg BD to expel the intestinal worm. He did not return for a follow-up appointment.

### Case presentation 2

A 43-year-old Aboriginal male presented to the Emergency department complaining of constipation for one month. He was triaged to the green zone as all his vital signs were stable. He had no previous medical illness. He was a farmer who worked within a densely forested area, spent extended periods living and working inside the jungle, and lived there for several days to weeks. While in the jungle, he sustained himself by consuming various insects, plants, other crawling creatures, and reptiles, including snakes.

The patient had an average body build and good hydration status upon examination. His vital signs were stable, with a blood pressure of 118/82 mmHg and a pulse rate at 80 bpm. The abdomen

was soft with no organomegaly. Figure 2 shows his chest (a) and abdominal (b) radiographs, which revealed multiple C-shaped opacities in the lungs bilaterally and in all quadrants of the abdomen. His blood investigations were all normal. A diagnosis of pentostomiasis was made, and the patient was discharged with oral albendazole 400mg bd.

## Discussion

Adult *Armillifer* spp. inhabit the respiratory tract of giant snakes. They produce large amounts of ova that are shed into the environment in snake feces and secretions. Intermediate hosts such as rodents ingest the ova, hatch, and larvae migrate to the viscera, encyst, and molt several times. The life cycle is completed when the snake ingests these rodents. Humans become accidental intermediate hosts after the uptake of environmental parasite ova from respiratory secretions or feces from the final hosts (giant snakes) or by consumption of contaminated snake meat [5].

Human pentastomiasis was documented in Peninsular and East Malaysia in the 1960s [4]. Pentastomid infection was discovered in a series of 30 consecutive autopsies conducted on Aborigines from five different states in West Malaysia; the most infected organs were the liver and the lungs [6]. Most cases are asymptomatic, usually found incidentally due to unrelated pathology [2]. Patients typically experience fever, abdominal discomfort, vomiting, diarrhea, jaundice, and tenderness in the abdomen. The organs most frequently afflicted are the liver, mesenteries, spleen, and lungs. Rarely do severe and potentially fatal cases occur, such as significant liver infections, mechanical ileus, and other forms of dissemination [7].

It is well known that snake meat consumption is a common practice in some parts of Southeast Asia. Risk factors for infection include consumption of undercooked contaminated snake meat and contact with live snakes and their secretions. Consuming river water tainted with snake

secretions is another way Malaysian aborigines could become infected [4].

Histopathological analyses are frequently necessary for a definitive diagnosis [3]. Recently, polymerase chain reaction (PCR) has emerged as a diagnostic tool. Still, its availability remains limited [8]. Diagnosis can be made radiologically when calcified nymphs of *Armillifer* spp. and less often, *L. serrata* are detected on the chest or abdominal radiographs, showing a horseshoe or C-shaped structures [2].

No treatment is required in asymptomatic patients. In symptomatic infections with heavy infestations, surgery is recommended to relieve obstruction and compressive symptoms. Pentastomiasis has no known effective antiparasitic chemotherapy; however, mebendazole, ivermectin, and praziquantel have been used in a few cases with apparent success [9,10].

Pentastomiasis in Malaysia is underreported but not uncommon. Cases often go undiagnosed due to nonspecific symptoms and lack of awareness. Primary healthcare providers play a crucial role in this disease's early detection and management. It is endemic in Malaysia, and snakes are essential in its transmission. To avoid infection, primary care providers should play a crucial role in diagnosing pentastomiasis and advising individuals, particularly snake handlers, to adhere to stringent preventive measures. Personal hygiene precautions are required to prevent human pentastomiasis [6]. These include boiling water before consumption and avoiding drinking river water. Authorities should provide health education to alert people about the risks of consuming undercooked snake meat, the possibility of transmission after handling snakes, and avoiding contact with snake excretions.

## Conclusion

In primary care setting, healthcare providers play a pivotal role in disseminating information and educating individuals about the prevention of human pentastomiasis by providing guidance on hygiene practices, offering advice on safe food

preparation methods, and emphasizing the importance of seeking medical attention promptly if symptoms and history suggestive of pentastomiasis arise. By fostering a proactive approach to prevention and early intervention, primary care providers can contribute significantly to reduce the incidence and impact of parasitic infection within the community.

### Acknowledgments

The authors thank the patient for agreeing to publish his case. We thank Hospital Tengku Ampuan Afzan for their outstanding services and dedication in managing the patient.

### Conflicts of interest

We have no conflicts of interest to disclose.

### Author contributions

Noor Emilia Emira wrote the first and final drafts. Abdul Hadi Said and Mubarak Mohd Yusuf reviewed, edited, and finalised the draft. All authors agreed with the results and conclusions.

### Ethics

Patients provided consent for the use of images and content for publication.

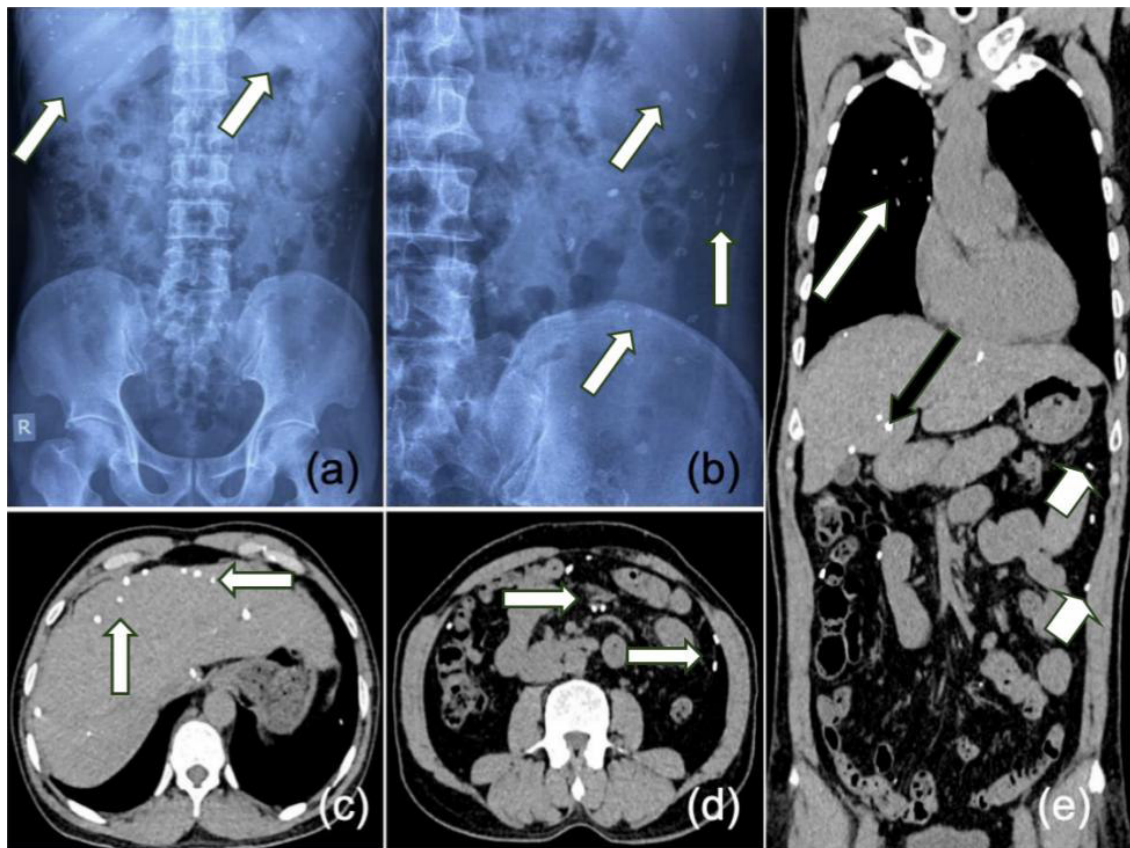


Figure 1. Abdominal radiograph (a) and an enlarged view of the left abdomen (b) showing numerous crescentic calcifications (arrows) scattered in the abdomen and pelvis. Plain CT abdomen in axial view reveals calcifications in the liver (c) and mesenteric fat (d). Coronal reconstruction CT image (e) showed calcifications in the lung (white arrow), liver (black arrow), and mesenteric fat (arrowhead).

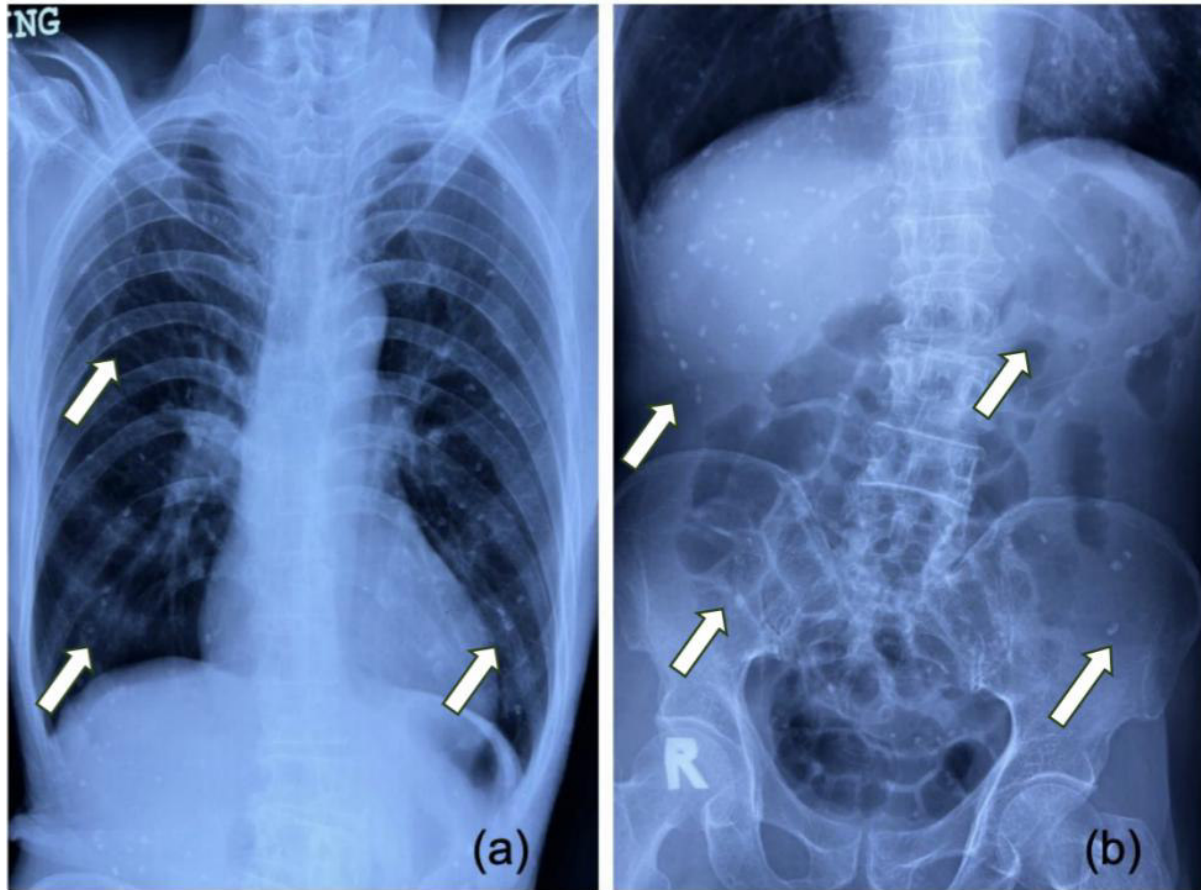


Figure 2. A chest (a) and abdomen (b) radiographs showed multiple C-shaped opacities in the lung and abdomen (arrow).

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## CASE REPORT

# Diagnostic Challenges of Pityriasis Lichenoides et Varioliformis Acuta (PLEVA).

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### Abstract

Pityriasis Lichenoides et Varioliformis Acuta (PLEVA) is an uncommon, inflammatory skin illness typified by erythematous papules that can mimic various dermatological and systemic conditions, making diagnosis difficult. We describe a case of a young adult who presented with generalized erythematous macules and crusted patches across the trunk that progressed to the extremities and scalp. A provisional diagnosis of guttate psoriasis with PLEVA as the differential diagnosis was made after a thorough history and examination. Further evaluation and skin biopsy confirmed the diagnosis of PLEVA through histopathological examination. The lesions subsided after two months of treatment with oral and topical corticosteroids.

**Keywords:** *Pityriasis lichenoides et varioliformis acuta, lichenoid dermatitis, primary care.*

## Introduction

Mucha-Habermann disease or Pityriasis lichenoides et varioliformis acuta (PLEVA) is a rare benign dermatosis with uncertain etiopathogenesis. It can affect individuals of any age but is most prevalent in children and young adults. PLEVA's diverse morphology and clinical presentation, which may resemble other dermatological conditions, often pose diagnostic challenges, and lead to delays in appropriate management. Here, we present the case of a patient who was initially diagnosed with guttate psoriasis, due to the similarity to Mucha-Habermann disease. However, the final diagnosis was PLEVA. This case highlights the importance of considering this uncommon condition in the differential diagnosis of dermatological disorders.

## Case presentation

A 21-year-old male presented with a vesicular eruption that had persisted for one month. Subsequently, recurrent erythematous macules and crusted patches appeared on his trunk, extended eventually to his extremities and scalp. The lesions were itchy, but spared his oral mucosa and nails. Initially, the patient thought he had the same condition as his nephew, who had been diagnosed with a varicella zoster infection. However, as the lesions persisted, he sought treatment at multiple clinics and was prescribed both systemic and topical antibiotics. Unfortunately, no improvement was seen despite the treatment.

Upon examination, his vital signs were stable, and he appeared well. Brownish scales were present throughout his skin, particularly on the trunk (Figures 1 and 2) and in all flexural areas. Newer erythematous macules, papules, and dried-up blisters with necrotic and occasionally hemorrhagic centers were observed, especially on the extremities (Figure 3). No alopecia, oral ulcers, joint tenderness, or nail involvement was seen. For laboratory investigations, the results were negative for the anti-nuclear antibody (ANA), syphilis, and viral screenings including Human immunodeficiency virus (HIV), Hepatitis

B, and Hepatitis C. Given the clinical diagnosis of guttate psoriasis and the need to rule out PLEVA, he was referred to the dermatology department. Histopathological examination (HPE) of his skin biopsy revealed subepidermal blisters with necrotic changes, ischemic epidermis, and marked lichenoid change with interface dermatitis (Figure 4). Spongiosis was also seen in viable epidermis, and focal epidermotropism was apparent (Figure 5). Despite all the immunofluorescence studies of the skin biopsy (IgG, IgA, IgM, and C3) negative for epidermal-dermal junction and blood vessels, PLEVA was still the diagnosis. The lesions resolved after two months of treatment with tapering doses of oral and topical corticosteroids.

## Discussion

Pityriasis lichenoides et varioliformis acuta (PLEVA), also known as Mucha-Habermann disease (MHD), is a rare cutaneous disorder that commonly occurs in children and young adults, with a slightly higher occurrence in males (56%) [1]. Although the exact origin of PLEVA is unknown, it is believed to be associated with an inflammatory response that is triggered by infectious agents [2]. PLEVA has been reported in cases following infections with pathogens like Epstein-Barr virus, HIV, varicella-zoster virus, herpes simplex virus type 2, Toxoplasma gondii, and Group A streptococcus [2]. Apart from that, PLEVA has been linked to certain medications such as antidepressants, statins, anti-tumor necrosis factor (anti-TNF), and various vaccines [2-5]. Moreover, T-cell dyscrasia-induced inflammation or immune complex-mediated hypersensitivity were suggested as potential causes of PLEVA [2]. In this case, the first theory is more plausible because he had exposure to varicella zoster infection from his nephew.

The hallmark features of PLEVA are characterized by the acute development of inflammatory papules and papulovesicular with hemorrhagic or necrotic crusts on the skin, usually on the trunk and flexural areas of the



extremities(1). While individual lesions may disappear within a few weeks, new groups of lesions frequently appear, leading to lesions at various stages of development. This can make diagnosing PLEVA difficult in a primary care setting. Rarely, patients have the severe variation of PLEVA known as febrile ulceronecrotic Mucha-Habermann disease (FUMHD) that may involve mucosal membranes, high fevers, and systemic complications like sepsis, splenomegaly, cardiomyopathy, and pulmonary involvement [2]. The present case report highlights two important points. Firstly, it underscores the challenges in diagnosing PLEVA in a primary care setting. PLEVA's clinical resemblance to other conditions and its rarity led to the initial misdiagnosis of diseases like guttate psoriasis, varicella zoster, pityriasis rosea, and secondary syphilis. Table 1 lists the clues that can help distinguish other diseases from PLEVA.

Secondly, there are challenges in delivering appropriate management for patients with PLEVA. Even though, PLEVA is usually a self-limiting disease, many patients experience itching, discomfort, and recurrence of their skin lesions, which can impact their quality of life. A recent systematic review recommended narrow-band ultraviolet B (UVB) phototherapy as the first-line therapy due to its high rate of complete remission. Oral erythromycin with or without topical corticosteroids, and low-dose methotrexate are recommended as second-line therapy [10]. In addition, methotrexate plays an important role in treating refractory PLEVA and FUMHD [2,4].

Acitretin, dapson, and cyclosporine may help as additional therapies for refractory PLEVA whereas for cases of FUMHD, systemic immunomodulators may be beneficial [2].

### **Conclusion**

This case highlights the diagnostic dilemma posed by PLEVA and its mimickers, where clinical presentation alone may not be sufficient for an accurate diagnosis. A high index of suspicion, coupled with histopathological examination, is essential for distinguishing PLEVA from other similar conditions and guiding appropriate management.

### **Acknowledgement**

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### **Conflict of interest**

All authors declare no conflicts of interest.

### **Authors contribution:**

- 1) Mohd Aizzuddin Abd Rahman: Writing, Editing, Literature Review, Supervision.
- 2) Nurjasmine Aida Jamani: Writing, Editing, Literature Review, Supervision.
- 3) Sarah Abdul Halim: Writing, Editing, Literature Review, Supervision.
- 4) Norsafina Zainun: Writing, Editing, Literature Review.



Figure 1. Generalized brownish scales with patches.



Figure 2. Generalized crusted lesions with central necrosis.



Figure 3. Erythematous macules and papules with dried-up blisters seen together with necrotic and a few hemorrhagic at the center of the crust.

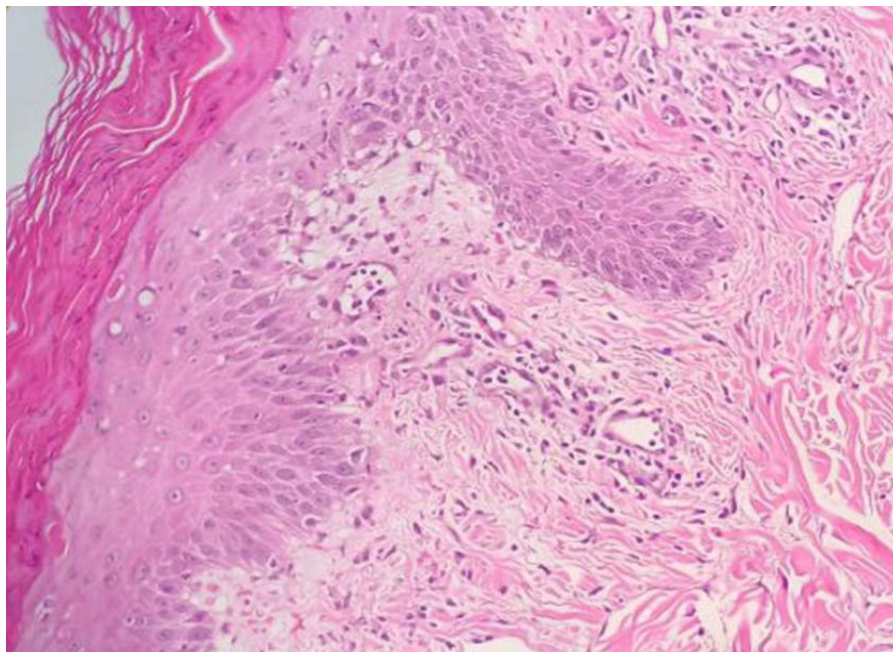


Figure 4. The section shows subepidermal blisters with lichenoid change and interface dermatitis. (Haematoxylin & eosin x100)

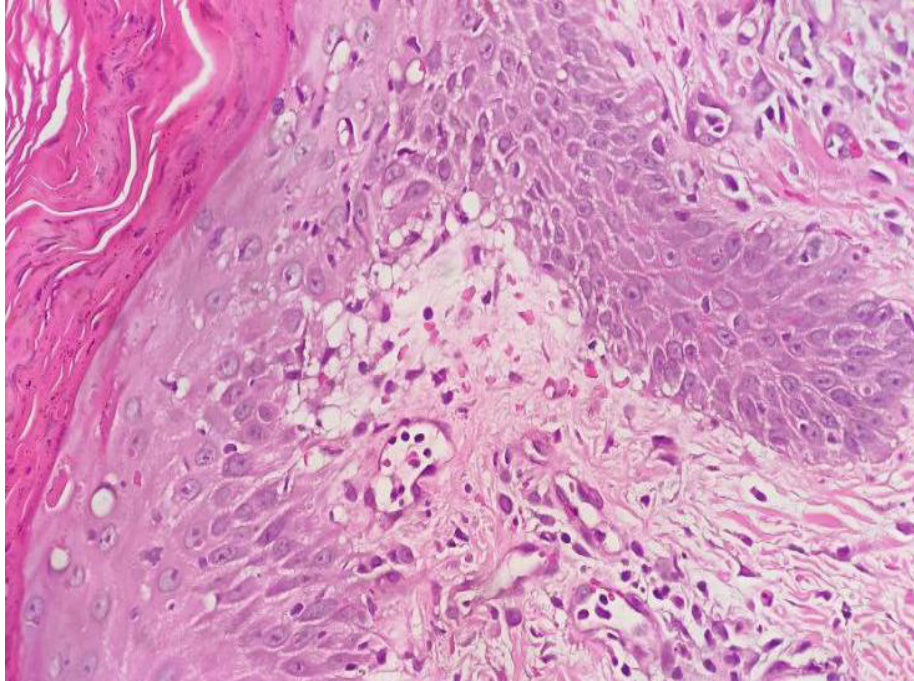


Figure 5: Focal epidermotropism is present. (Haematoxylin & eosin x 200)



Table 1. Clues that help distinguish other diseases from PLEVA.

Disease	Clinical Findings on Skin	Diagnostic test	Treatment strategies
Guttate psoriasis	Guttate psoriasis appears as tiny, erythematous papules and plaques, similar to PLEVA, but may also involve nails (6).	Clinical diagnosis	a) Narrowband UVB Phototherapy b) Topical corticosteroids as adjunct
Varicella	Varicella is frequently likened to a dewdrop on a rose petal and typically manifests as crusted lesions on the sixth day of the illness, resembling PLEVA. Nevertheless, the duration of the disease is briefer than PLEVA and normally lasts for two weeks (7).		a) Self-limited disease b) Symptomatic treatment c) Intravenous antiviral therapy is indicated in complicated cases of varicella.
Pityriasis rosea	The herald patch, "Christmas tree" pattern distribution, and collarette of scale are key features that distinguish pityriasis rosea from PLEVA. (8).		a) Self-limited disease b) Symptomatic treatment
Secondary syphilis	Classically, the rash is a generalized, non-itchy, and symmetrical macular or papular eruption that affects the entire body, including the palms and soles (9).	Specific treponemal tests (TPHA/TPPA/EIA)	a) Intramuscular Benzathine penicillin 2.4 mega units in a single dose

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## CASE REPORT

# Diagnosing the Rare: Clinical Experience in Acardia Twin Recognition - A Case Report.

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### Abstract

Twin Reversed Arterial Perfusion (TRAP) sequence or acardiac twin, is a rare condition in monochorionic twin pregnancies, presenting significant diagnostic and management challenges. Early detection of TRAP is crucial to improve foetal outcomes, particularly for the pump twin. We report the case of a 37-year-old gravida 5, para 3 + 1, who was initially misdiagnosed with a singleton pregnancy based on clinical and ultrasound examinations conducted at both primary and secondary health facilities. A marked discrepancy between the symphysiofundal height and ultrasound parameters later led to reassessment, revealing a twin pregnancy complicated by TRAP sequence. Radiofrequency ablation (RFA) was successfully performed on the acardiac twin at 28 weeks. The pregnancy ended at 30 weeks gestation with preterm birth, in which the pump twin was delivered alive while the acardiac twin was stillborn. This case highlights the importance of vigilant antenatal assessment, timely diagnosis, and intervention to optimise outcomes in TRAP sequence pregnancies.

**Keywords:** *Acardiac twin, antenatal diagnosis, fetal outcome, monochorionic twin pregnancy, Twin Reversed Arterial Perfusion (TRAP) sequence.*



## Introduction

Twin Reversed Arterial Perfusion (TRAP) sequence is a rare condition that only occurs in monochorionic twin pregnancies. This condition is characterised by the presence of one twin with an underdeveloped or absent heart (acardiac twin), perfused by its co-twin (pump twin) through placental arterial anastomoses. The incidence of TRAP sequence has traditionally been estimated at 1 in 35,000 pregnancies, or 1 in 100 monozygotic twin pregnancies. However, with advances in ultrasound diagnostics and the increasing use of assisted reproductive technologies, the incidence appears to be rising [1]. Accurate diagnosis and early intervention are vital to improve foetal outcomes, particularly for the pump twin, which is at risk of significant morbidity and mortality.

## Case report

A 37-year-old woman, gravida 5, para 3 + 1, with underlying beta-thalassemia trait, presented to the health clinic with a history of amenorrhea for 5 months. She reported experiencing foetal movements and sought antenatal care. This was the first pregnancy with her second husband, who had a first-degree family member with twins. It was a non-consanguineous marriage. At booking, she was asymptomatic and well. No prior ultrasound had been performed. Physical examination revealed a gravid uterus corresponding to 22 weeks of gestation. An obstetric abdominal ultrasound examination showed a singleton foetus at 21- 22 weeks with no apparent abnormalities. The patient confirmed the accuracy of her menstrual dates and denied using any contraceptive medication. A follow-up ultrasound was arranged to confirm the gestational age. However, three days later, she was admitted to the hospital with maternal pyrexia, likely due to influenza. The initial obstetric evaluation by the O&G team during admission again revealed a singleton foetus with no detected abnormalities. However, prior to discharge, a significant discrepancy between the

gestational age estimated by clinical symphysiofundal height (28 weeks) and the ultrasound parameters (singleton foetus corresponding to 22 weeks), led to a consultation with an obstetrician. A careful ultrasound examination by the obstetrician revealed a twin pregnancy with no separating membrane, one normal twin, and another twin showing no head and thoracic cavity with multiple cystic lesions in the abdominal cavity (Figure 1), consistent with twin reversed arterial perfusion (TRAP) sequence. The patient was referred to the maternal foetal medicine unit for further management. Following detailed counselling, the patient consented to radiofrequency laser ablation (RFA) at 28 weeks gestation. Post-procedure doppler ultrasound confirmed the absence of blood flow to the acardiac twin. At 30 weeks, cervical funneling by ultrasound warranted admission for intravenous magnesium sulphate and corticosteroids, followed by an emergency lower segment caesarean section. The pump twin was delivered weighing 1.41 kg with APGAR scores of 8 and 9 at 1 and 5 minutes, respectively. The acardiac twin, weighing 0.5 kg, was stillborn, with post-mortem findings confirming the malformations observed on the ultrasound. The surviving twin was later discharged in stable condition at 2.1 kg after three weeks in the neonatal intensive care unit.

## Discussion

Twin reversed arterial perfusion (TRAP) sequence, also known as acardiac twin, is a rare condition exclusively happens in monochorionic twin pregnancies, with a prevalence estimated between 1 in 9,500 to 1 in 44,000 births [2]. In TRAP, abnormal vascular connections between the twins lead to the development of an acardiac twin. This case illustrated the acardiac acephalus form, the most common type of acardiac twin, where the foetus displays no cranial development as well. Early diagnosis is critical as delayed detection of the TRAP sequence may result in adverse outcomes for the pump twin. Doppler

ultrasound is able to detect reversed blood flow in the acardiac twin as early as the first trimester. Advanced imaging like MRI in the second trimester can further confirm the diagnosis [3]. Early intervention in the TRAP sequence significantly improves the prognosis of the pump twin. Without appropriate and timely intervention, the burden on pump twin's cardiovascular system increases as the acardiac twin grows, leading to the risks of heart failure, foetal hydrops, and intrauterine death. [4] There are several interventions that could improve the prognosis of the pump twin, with radiofrequency ablation (RFA) and intrafoetal laser (IFL) being the two commonly preferred techniques, alongside expectant management. As demonstrated in this case, RFA has shown to have better outcomes, with higher live birth rates and lower rates of foetal complications and intrauterine death compared to IFL and expectant management. Specifically, the live birth rate from RFA was 70.6% compared to 66.7% with expectant management [5]. The optimal timing for intervention is still debated, but early treatment has been proposed to reduce first-trimester demise and improve overall outcomes [6]. This case highlights the complexity of diagnosing TRAP sequence, particularly when early ultrasound even fails to detect the twin pregnancy. Despite the late diagnosis, successful management through RFA at 28 weeks ensured the survival of the pump twin. Although the pregnancy was complicated by preterm delivery, the outcome for the foetus has been favourable.

## **Conclusion**

The presented case underscores the diagnostic and management challenges of TRAP sequence, particularly the importance of thorough clinical and ultrasonography evaluations when discrepancies in gestational age arise. While early

detection is key to optimizing foetal outcomes, this case demonstrates that effective management can still result in favourable outcomes for the pump twin, even with a late diagnosis.

## **Acknowledgement**

The authors would like to thank the patient for her permission and cooperation in the preparation of this case report.

## **Conflict of interest**

There is nothing to declare.

## **Patients' consent for the use of images and content for publication**

The patient gave written consent to use the image and the case for publication.

Authors' contribution

Siti Dzulaikha Zulkifli: Writing, editing, and literature review.

Nor Azam Kamaruzaman: Writing, editing, literature review and supervision.

Mohd Daud Che Yusof: Editing and supervision.

## **What is new in this case report compared to the previous literature?**

This case report emphasizes the crucial role of precise clinical correlation between symphysiofundal height measurement and obstetric ultrasound findings during antenatal examinations, in arousing clinical suspicion of a twin pregnancy.

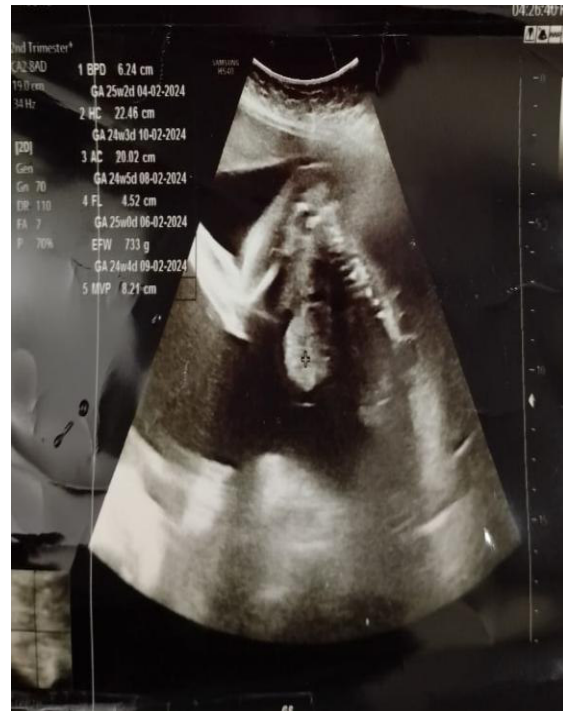
It's important to recommend practices that could increase early detection rates, such as increased training for sonographers on the specific ultrasound markers of the TRAP sequence and other rare twin complications.

## **What is the implication to patients?**

Delayed detection of the twin pregnancy especially TRAP sequence may result in adverse outcomes to the pump twin.



1(a)



1(b)

Fig. 1(a) and 1(b). Ultrasound findings of the acardiac twin. Figure 1(a) Transverse view: multiple dilated bowels in the abdominal cavity. Figure 1(b) Sagittal view: malformed foetus with no head and poorly developed trunk with omphalocele, the lower limbs were well formed.

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## CASE REPORT

### **A Journey of Hope: A Primigravida with an Ovarian Teratoma.**

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#### **Abstract**

Ovarian teratomas (OTs) are categorized as either mature or immature. Mature cystic teratomas (MCTs), also known as dermoid cysts, are the most common type of germ cell tumours in the ovaries of women of reproductive age. OTs generally remain asymptomatic until they reach a substantial size. Transvaginal and transabdominal ultrasounds are crucial for evaluating pelvic masses and distinguishing between benign and malignant cases. These imaging methods are commonly used for diagnosis. A histopathology report is essential for confirming an OT diagnosis, and surgical intervention, using various approaches, is the only treatment option. In this case, we reported a primigravida woman at 8 weeks of gestation presented with irregular menses and ultrasound imaging revealed a unilateral dermoid cyst, which was subsequently diagnosed as a mature ovarian teratoma.

**Keywords:** *Dermoid cyst, mature cystic teratomas(MCTs), ovarian teratomas(OTs).*

## Introduction

The occurrence of ovarian tumours during pregnancy is relatively rare, with an incidence between 0.3% and 5.4%. Among these, dermoid cysts and cystadenomas are the most prevalent benign ovarian tumours found [1]. Ovarian teratomas can be classified into several subcategories: mature cystic teratomas, immature teratomas, monodermal (highly specialized) teratomas (such as struma ovarii, carcinoid tumours, neuroectodermal tumours, and sebaceous tumours), and fetiform teratomas [2]. The most common clinical manifestation of patients experiencing MCT is asymptomatic, however, it can sometimes cause major abdominal and pelvic pain [2]. Early detection and detailed ultrasounds are vital in antenatal care within primary care settings, ensuring the health of both the expectant mother and her growing foetus.

## Case report

A 23-year-old primigravida at 8 weeks of gestation came for antenatal booking. She did not have a previous medical or surgical history. She sought antenatal care after she missed her period for 8 weeks. She also complained of having irregular menses over the past few months. During her initial presentation, she informed that she had irregular menses but was never investigated before. Her menstrual cycles occurred every 2 to 3 months, lasted for 3 days, and were not accompanied by dysmenorrhea. Otherwise, she had no nausea, vomiting, abdominal pain, abnormal uterine bleeding, or vaginal discharge. There was no excessive hair growth on her face or body. Otherwise, she was well with no gastrointestinal or genitourinary symptoms. She had no prior history of illnesses or allergies and denied using any medications. On physical examination, she was alert, not pale, and her vital signs were within the normal range. An abdominal examination was unremarkable. Her urine pregnancy test was positive. Haemoglobin at booking was 11.9 g/dL. All antenatal infectious screenings were non-reactive.

The ultrasound demonstrated a viable singleton foetus with a crown-rump length of 17.9 mm, indicating an 8-week gestation period [Figure 1]. However, there was an incidental finding of a large right adnexal mass measuring 4.6 cm × 4.1 cm, most likely originating from the right ovary [Figure 2]. The mass was heterogeneous in nature and there was a presence of an echogenic focus with acoustic shadowing within a predominantly cystic structure. This was highly suggestive of an ovarian teratoma. She was then referred to the obstetrics and gynaecology team for confirmation of the findings and further expert management. A diagnosis of a right dermoid cyst was established at 8 weeks of gestation, and she underwent a laparoscopic right cystectomy. Histopathological examination confirmed the cyst as a mature cystic teratoma. The histopathological findings revealed a diverse range of well-differentiated tissues originating from all three germ cell layers, each in its mature form [Figure 3 (a-d)]. Tumour markers, including CA-125 and alpha-fetoprotein, were within normal limits. Postoperatively, her antenatal care resumed as normal.

## Discussion

Dermoid cysts, or mature cystic teratomas, are typically found in young women of reproductive age and represent 20%-40% of ovarian masses in pregnant women [1]. Cystic teratoma is the most common ovarian neoplasm, consisting of well-differentiated derivatives of the germ cell layers (ectoderm, mesoderm, and endoderm) that can develop into structures like hair, muscle, teeth, or bone. Teratomas are tumours made up of various parenchymal cell types from more than one germ layer, often including all three. They originate from totipotential cells and are usually located midline or paraxial [3]. The ovary is the most common gonadal site, though they are also found in the testes. Occasionally, cystic teratomas appear in sequestered midline embryonic cell rests, such as the mediastinum (7%), retroperitoneum (4%), cervical region (3%), and

intracranial area (3%) [4]. These cells can differentiate into various tissues found in the body, including hair, teeth, fat, skin, muscle, and endocrine tissue. Most MCTs are unilateral, although they can also be bilateral [5]. In unilateral scenarios, MCT is more common on the right side (72.2%). Menstrual irregularities are also linked with 15% of cases. Although many MCTs are discovered incidentally, about 20% of cases necessitate clinical intervention due to complications. In this patient, the presenting symptom was only irregular menstruation, without abdominal or pelvic pain, however, it is still leading to a diagnosis of mature ovarian teratoma.

Ovarian torsion is rare during pregnancy as the gravid uterus usually limits the space for the cyst to twist on its pedicle [6]. Ovarian torsion resulting from a mature cystic teratoma (MCT) during pregnancy can lead to acute abdominal pain, necessitating emergency surgery to prevent damage to the ovary. Both torsion and rupture of an ovarian teratoma during pregnancy can cause serious complications and negative outcomes.

Ovarian torsion occurs when the suspensory ligament, which connects the ovary and its vascular peduncle to the pelvic wall, twists. This twisting leads to gradual swelling of the ovarian tissue, reduced blood flow (ischemia), and ultimately, haemorrhagic infarction. Additionally, severe tissue damage or death may result in the loss of the affected ovary [6]. Besides that, rupturing an ovarian teratoma can cause severe abdominal pain, internal bleeding, and peritonitis, all of which require immediate medical attention. In pregnant women, this can lead to severe pain, bleeding, and shock [7]. Detecting free fluid in the abdomen via ultrasound can indicate a rupture. Such ruptures during pregnancy can significantly affect outcomes, potentially causing shock or haemorrhage and necessitating emergency surgery. Although the overall prognosis is generally good, complications can still arise for both mother and baby. Research has shown that mature cystic teratomas (MCTs) are associated with adverse pregnancy outcomes like

intrauterine growth restriction (IUGR), preterm deliveries, and complicated labour [7]. Specifically, MCTs larger than 5 cm increase the risk of premature rupture of membranes (PROM). The increased use of ultrasonography (USG) in recent years has led to the diagnosis of asymptomatic multiple ovarian masses in pregnant women [6]. Ultrasound is critical in identifying ovarian masses and differentiating between benign and malignant tumours. About this case, a bedside ultrasound at a primary care setting revealed a unilateral ovarian cystic mass which was later confirmed as ovarian teratoma. Diagnosing ovarian tumours is challenging because of their diverse internal structures and appearances on ultrasound. Echogenic, fluid-filled masses can look like solid lesions, making it hard to identify mature cystic teratomas (MCTs). Ovarian cystic teratomas can display a wide range of echo patterns [8]. In this case, the mass appeared mixed, with an echogenic area and shadowing inside a mostly cystic structure, which supports the diagnosis of an ovarian teratoma.

While MRI provides detailed tissue plane definitions and relationships with other organs when ultrasound results are inconclusive [9], it was unnecessary here as ultrasound findings alone identified the dermoid cyst, later confirmed as a mature ovarian teratoma. Laparoscopy is the preferred treatment approach, with cystectomy being ideal to preserve ovarian parenchyma. This patient underwent a laparoscopic right cystectomy to remove the unilateral dermoid cyst. Research has shown that laparoscopic removal of ovarian dermoid cysts is safe [10], and it is considered safer than laparotomy during the late first or second trimester, despite the risk of cyst rupture [11]. The same approach was used in this case.

## Conclusion

Mature teratoma is a benign tumour with a good prognosis. Diagnosis is presumed by radiological examinations and confirmed by pathology. However early diagnosis is crucial with a good

skill in antenatal scan in primary care so that it will not be missed and can be managed successfully and in a timely manner.

Recognizing both uncommon presentations and typical signs, alongside a fundamental understanding of their pathological counterparts, enables more accurate diagnosis and appropriate treatment decisions. Ultimately, the patient's clinical symptoms should guide the choice of the most suitable management approach.

### Acknowledgments

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**Conflict of interest** The authors declare that they have no conflict of interest.

### Patient's consent for the use of images and consent for publications

The patient provided consent to use images and content for publications.

### Authors' Contribution

- Dr. Norasikin: Contributed to the conception of the study and drafting of the case report.
- Dr. Fatin 'Amira : Analyzed and assisted in histopathological interpretation.
- Dr. Mohd Daud : Provided insights into the discussion and reviewed the literature on ovarian teratoma effects during pregnancy.
- Dr. Suhaiza : Reviewed the manuscript and provided critical revisions.
- All authors : Read and approved the final manuscript.



Figure 1. Ultrasound picture at 8 weeks of gestation showed a singleton viable



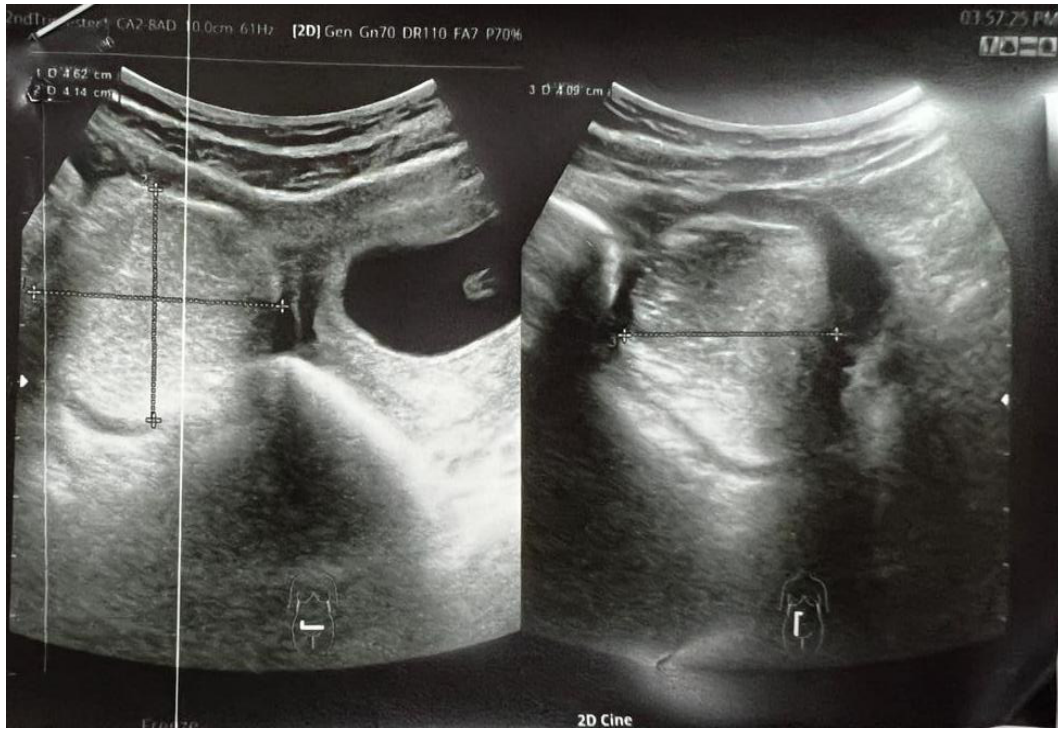


Figure 2. Ultrasound picture at 8 weeks gestation showed one large heterogenous echogenicity cystic mass size of 4.6 cm x 4.1 cm

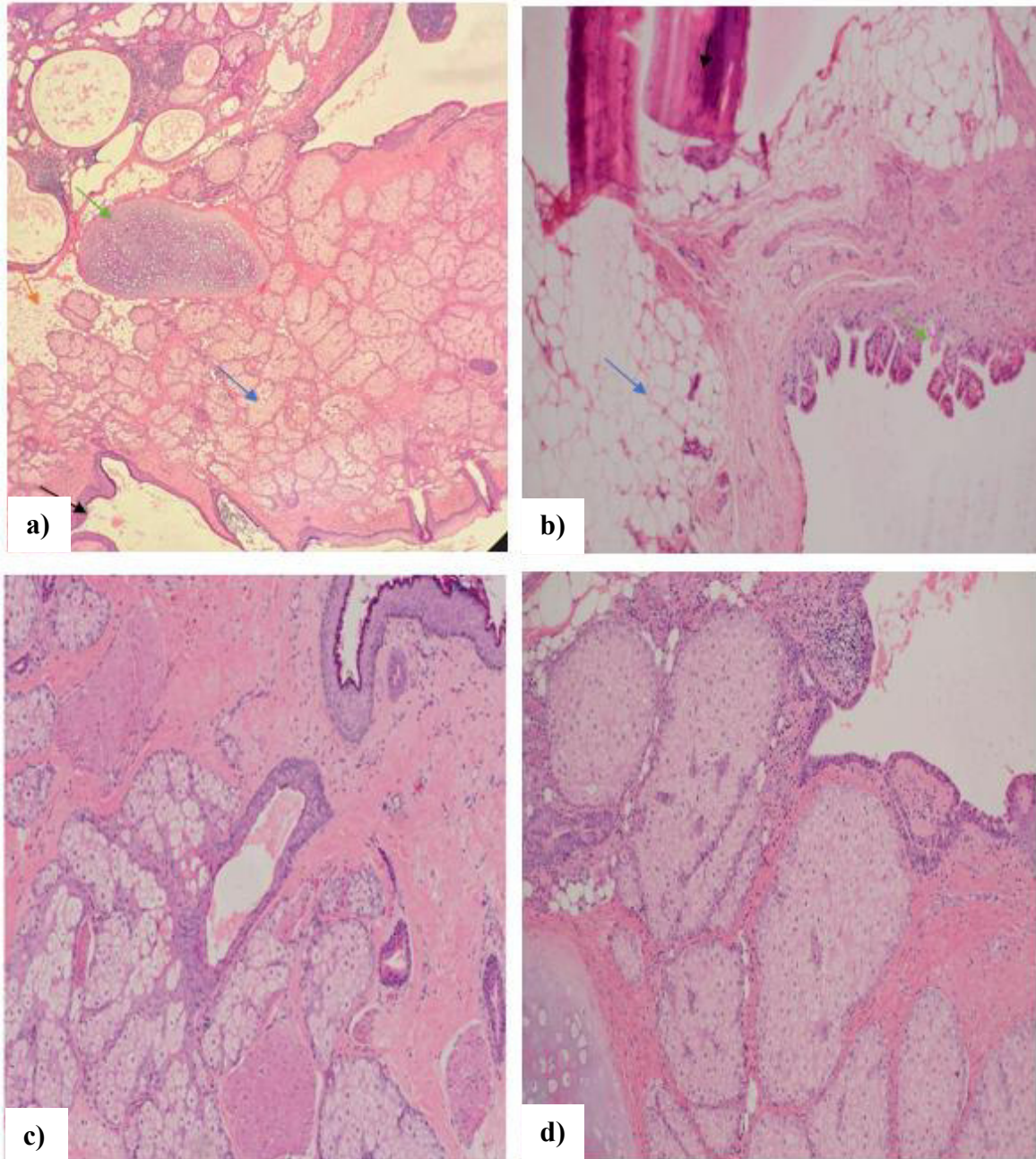


Figure 3. (a) Hematoxylin and Eosin staining at 40X show view of mature cystic teratoma, composed of stratified squamous epithelium (black arrow), sebaceous glands (blue arrow), mature cartilage (green arrow) and mature adipose tissue (red arrow). (b) Hematoxylin and Eosin staining at 100X show mature bone (black arrow), mature adipose tissue (blue arrow) and choroid plexus (green arrow). (c) Hematoxylin and Eosin staining at 100X show stratified squamous epithelium, skin adnexae and smooth muscle bundles. (d) Hematoxylin and Eosin staining at 100X show pseudostratified columnar epithelium with goblet cells, sebaceous glands and mature cartilage.

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## CASE REPORT

### Warthin Tumour: A Case Report and Approach in Primary Care.

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#### Abstract

Warthin tumour (WT) is an infrequent benign salivary gland neoplasm, that primarily occur in the parotid gland, the largest of the salivary glands. Comprising roughly 15% of all salivary gland tumours, these lesions are the second most prevalent type of salivary gland neoplasm after pleomorphic adenoma encountered in clinical practice. Here, we report a case of Warthin tumour of six years' duration in a 46-year-old male patient in the left parotid gland and will discuss the diagnostic process, differential diagnoses, and management of a suspected WT in a primary care setting. While Warthin tumours are generally considered to be non-malignant, they can mimic carcinomas in terms of their glandular size and appearance. As such, it is crucial for primary care providers to be able to recognize and appropriately manage these lesions.

**Keywords:** *Parotid gland swelling, primary care, salivary gland neoplasm, Warthin tumour.*

## Introduction

Warthin tumours (WT), also known as papillary cystadenomas, are a type of benign tumour that commonly arises in the parotid gland, the largest of the salivary glands.[1] Synonyms for WT are adenolymphoma, cystadenolymphoma and papillary cystadenoma lymphomatosum. These tumours are characterized by a papillary or adenomatous proliferation of oncocytic epithelial cells, accompanied by a prominent lymphoid stroma. Parotid swelling can have a variety of aetiologies, ranging from benign to malignant conditions.[2] A careful assessment of the patient's symptoms, physical examination findings, and any underlying medical conditions is essential in guiding the diagnostic and management plan. A multidisciplinary approach involving primary care, otolaryngology, and radiology is recommended to ensure a comprehensive evaluation and appropriate management. Clinicians should perform a thorough history and physical examination, assessing the location, size, consistency, and any associated symptoms. Imaging studies, such as CT or MRI, may be ordered to further characterize the lesion and guide the need for biopsy or surgical intervention. [3,4]

## Case report

In this case report, we present a 46-year-old male who was a heavy smoker with more than 40 pack-years, and no other co-morbidities and presented to his primary care physician with a gradually enlarging, painless swelling of the left side of his face. The patient first noticed the swelling about 5 years prior but had refrained from seeking medical attention, as he was concerned that the mass could potentially be indicative of a malignant process. He denied a history of trauma to the area, fever, loss of appetite or weight loss and there was no family history of malignancy. On physical examination, a firm, non-tender, mobile mass sized about 6 x 5 cm in diameter was palpated in the left parotid region. There was no palpable lymphadenopathy or any other concerning findings. Given the patient's age and

presentation, the primary care provider considered the differential diagnosis of Warthin tumour, pleomorphic adenoma, and salivary gland carcinoma[5,6]

The physician counselled the patient on the importance of further evaluation and possible surgical treatment to remove the mass. The patient was hesitant at first, as he was concerned about potential complications and the impact on his appearance. Recognizing the patient's apprehension, the physician took the time to thoroughly discuss the diagnostic process, which would likely involve imaging studies such as an ultrasound or CT scan to further characterize the mass, and a biopsy to confirm the diagnosis. The physician acknowledged the patient's cosmetic concerns and emphasized that modern surgical techniques have advanced significantly, often allowing for the removal of parotid gland tumours with minimal visible scarring and preservation of the patient's natural facial appearance. After carefully weighing the risks and benefits, the patient agreed to proceed with the recommended evaluation.

The mass was subsequently evaluated with a fine-needle aspiration (FNA) biopsy, which revealed a smear showing a few small cohesive sheets of oncocytes displaying abundant granular cytoplasm, central round nucleus with prominent nucleolus with numerous lymphocytes and granular debris in the background (Figure 1 & 2). However, no atypical cells or malignant cells were seen. The fine-needle aspiration of the left parotid swelling is consistent with the diagnosis of Warthin tumour. Given the benign nature of the tumour, the patient was referred to a head and neck surgeon for surgical excision. A CT scan of the neck was done as a preoperative assessment with the finding of a large left parotid solid cystic mass arising from the lower pole of the superficial lobe of left parotid gland measuring about 5.4 x 6.1 x 9.1 cm (AP x W x CC) (Figure 3 & 4). The patient opted for surgical excision, and the mass was successfully removed without complication. Histopathological analysis confirmed the diagnosis of Warthin tumour. During the



subsequent follow-up visits, the patient's surgical site healed well, and he reported no significant postoperative complications.

## Discussion

In the context of primary care, the evaluation and management of Warthin tumour (WT) present unique considerations, as early detection and appropriate referral can significantly impact the patient's clinical trajectory. Warthin tumour, a relatively uncommon yet benign neoplastic growth, is characterized by its propensity to develop within the salivary glands, with a notable tendency to primarily affect the parotid gland. In this case report, we delve into the nuances of Warthin tumour, exploring its prevalence, clinical presentation, and the primary care approach of diagnosing and management. Additionally, we provide a comparative overview of Warthin tumour and other common salivary gland tumours mentioned in the Table 1.

Warthin tumour (WT) typically appears as a painless, slow-growing, round or oval mass that is well-circumscribed and usually located in the lower pole of the parotid gland, though it can occasionally present as a fluctuant swelling. The tumour's borders may become indistinct, often due to secondary inflammation. Tumour sizes generally range from 2 to 4 cm, but larger tumours up to 12 cm have been reported. The average duration of symptoms is about 21 months, with pain occurring in approximately 9% of patients. Other symptoms can include earache, tinnitus, and deafness, while facial paralysis is uncommon. When pain or facial paralysis does occur, it is often linked to the metaplastic variant of WT or secondary inflammation and fibrosis. There is a strong association between WT and cigarette smoking, with smokers being about eight times more likely to develop WT than non-smokers. Other contributing factors may include radiation exposure and autoimmune disorders. WT typically affects elderly individuals, most commonly in their 50s and 60s, with an average age of 62 years. It is rarely seen in individuals

under 40. WT occurs in both Caucasians and Asians, but it is very rare in African Americans and Black Africans. The condition also shows a male predominance, with a male-to-female ratio of 2.3:1. [1][2]

Recognizing the need for a comprehensive evaluation and the likelihood of a Warthin tumour, the primary care physician promptly referred the patient to an otolaryngologist, who could provide the necessary diagnostic workup, including imaging studies, and form an appropriate treatment plan. Fine-needle aspiration cytology (FNAC) had a high diagnostic accuracy in the diagnosis of Warthin tumour, with a sensitivity of 93%, while the specificity was 94.8% and the accuracy, of 94.6% [6][7]. The primary care physician's recognition of the characteristic clinical presentation, combined with the confirmation of the diagnosis through appropriate testing, is critical in guiding the patient towards the most suitable management approach.

The multifocal nature of Warthin tumours and their tendency to recur after incomplete surgical removal underscores the critical importance of a thorough preoperative assessment and the selection of an appropriate surgical approach to optimize treatment outcomes and minimize the risk of potential recurrence in the future [8]. Careful follow-up is also essential, as the literature has reported cases of malignant transformation and the development of cervical lymph node metastases in some instances.[6][7] In the case of the patient presented, the otolaryngologist performed a complete superficial parotidectomy, which is the recommended surgical intervention for Warthin tumours, as it allows for the complete excision of the affected gland and reduces the likelihood of recurrence [9].

The primary care physician's role in the management of Warthin tumour extends beyond the initial diagnosis, as they are responsible for coordinating the patient's care, providing ongoing support, and monitoring for any potential complications or recurrence during the follow-up period. This case demonstrates the pivotal role of

the primary care physician in recognizing uncommon salivary gland pathologies, effectively communicating the diagnostic and treatment plan to the patient, and coordinating timely referral to the appropriate specialist even in the setting of patient anxiety and delayed presentation to ensure appropriate diagnosis and treatment.[10]

### **Conclusion**

Prompt recognition and appropriate management of parotid swelling are crucial to avoid complications and ensure the best possible patient outcomes. The primary care provider's role in this process is essential, as they are often the first point of contact for patients with this condition. By working collaboratively with specialists, the primary care provider can ensure a comprehensive and effective approach to managing parotid swelling. By maintaining a high index of suspicion for Warthin tumour in patients presenting with a painless parotid mass, primary

care providers can facilitate early detection and timely referral to the appropriate specialists, ultimately improving patient outcomes and quality of life.

### **Conflict of Interest**

There was no conflict of interest.

### **Source of financial/funding**

None

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### **Authors contribution**

All authors contributed to the drafting of the manuscript, data collection, analysis and editing the manuscript.

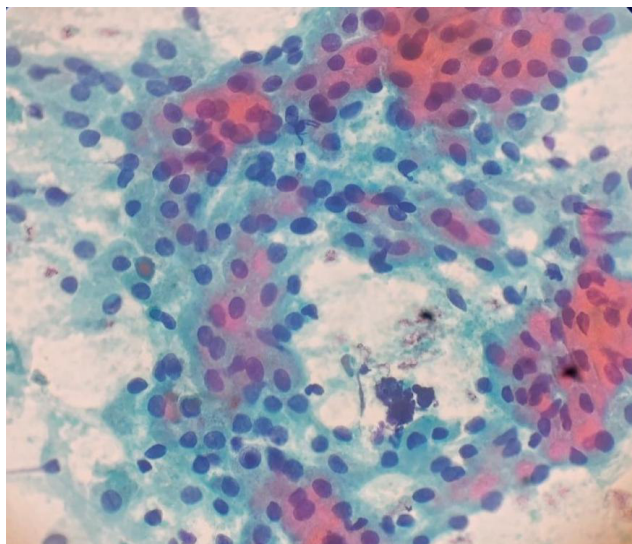


Figure 1. Cohesive groups of oncocytic cells (40x magnification)



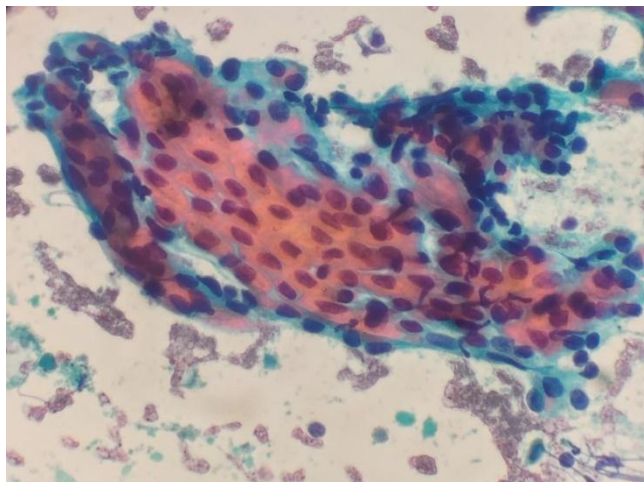


Figure 2. Groups of oncocytic cells with lymphocytes in the background (40x magnification)

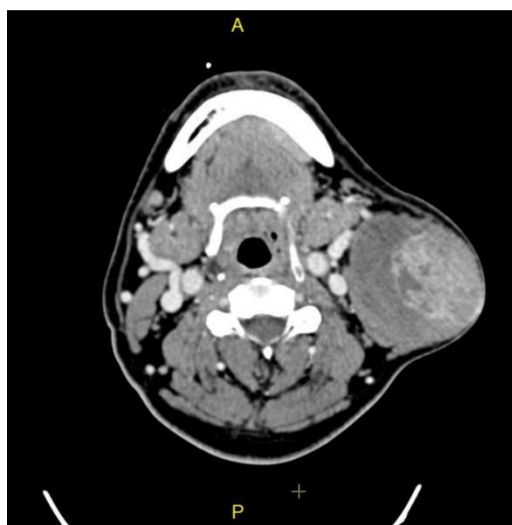


Figure 3. CT scan of the neck with contrast of the patient demonstrating large left parotid solid cystic mass in axial view



Figure 4. CT scan of the neck with contrast of the patient demonstrating large left parotid solid cystic mass in coronal view

Table 1. This table provides a comparative overview of the key features of Warthin's tumour and other common salivary gland tumours. [1,2,5,6]

<b>Feature</b>	<b>Warthin's Tumour</b>	<b>Pleomorphic Adenomas</b>	<b>Mucoepidermoid Carcinoma</b>	<b>Adenoid Cystic Carcinoma</b>
Prevalence	15%, second most common benign salivary gland tumour	45% - 70%, most common benign salivary gland tumour	30%, most common malignant salivary gland tumour	10%, second most common malignant salivary gland tumour
Nature	Benign	Benign	Malignant	Malignant
Location	Primarily parotid gland	Primarily parotid gland, can occur in other glands	Parotid gland, minor salivary glands	Parotid gland, minor salivary glands
Gender Predilection	Male predominance	Equal in males & females	Slightly female predominance	Equal in males & females
Symptoms	+ Slow-growing mass + Painless	+ Slow-growing mass + Painless + Facial nerve involvement (if the size is large/undergoes malignant change)	+Rapid growth +Painful +Facial nerve involvement	+Slow-growing mass +Painful +Facial nerve involvement
Histology	Cystic spaces, lymphoid stroma, oncocytic epithelium	Mixed epithelial and myoepithelial cells with stromal component	Mix of mucous, intermediate, and epidermoid cells	Cribriform, tubular, solid patterns
Treatment	Surgical excision	Surgical excision	Surgical excision, possible adjunct therapy (radiation/chemotherapy)	Surgical excision, radiation therapy
Prognosis	Excellent	Excellent	Variable, depends on grade and stage	Guarded, with frequent recurrences and potential distant metastasis

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## CASE REPORT

# Recurrent Haematuria in a Toddler: A Journey from Suspected UTI to Precocious Puberty.

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### **Abstract**

Numerous factors can contribute to the presence of blood in the urine. While the causes of gross haematuria often suggest a urological origin, it may also result from gynaecological pathology contaminating the urine. We present a case of a 2-year-2-month-old girl who presented with recurrent haematuria, her third episode in five months. Her previous two episodes were treated as urinary tract infections (UTI). One week before this most recent presentation, she had an upper respiratory tract infection, which resolved with a course of antibiotics. Although she was normotensive, she was referred to a tertiary centre for suspected post-streptococcal glomerulonephritis, based on urinalysis findings of erythrocytes 3+, trace proteinuria and presence of leukocytes. At the hospital, she was initially treated with antibiotics for probable UTI. However, persistent fresh blood staining her diaper prompted a second genital evaluation, which revealed blood seeping from the vagina. Further assessment showed signs of puberty suggesting that menstruation was contaminating the urine sample. This case report highlights the need for clinicians to consider a broad differential diagnosis when evaluating haematuria in paediatric patients and the importance of thoroughly assessing secondary sexual characteristics in young girls with haematuria to facilitate early recognition of precocious puberty.

**Keywords:** *Gross haematuria; post-streptococcal glomerulonephritis; precocious puberty.*

## Introduction

Haematuria, a common occurrence in children, can be seen either macroscopically or microscopically. Gross haematuria may manifest as red, pink, brown or dark urine. While the causes of macroscopic and microscopic haematuria may overlap, gross haematuria often points towards a urological origin, whereas microscopic haematuria is more commonly associated with glomerular issues [1]. While most cases of gross haematuria in the paediatric age group are typically linked to urinary tract sources, in a girl, the blood may originate from the genital tract. Therefore, a thorough evaluation of the genitourinary system is crucial in cases of haematuria to avoid overlooking external injuries to the urethra or vagina, such as those resulting from physical or sexual abuse, or, in rare instances, to prevent overlooking menstruation as the cause.

Precocious puberty (PP) in females occurs when pubertal signs appear before the age of eight, with breast development being the earliest manifestation [2]. Other than advanced thelarche and pubarche, menarche is one of the most common presentations of PP [3,4]. A study in Korea reported that the overall incidence of central precocious puberty in girls was 15.3 per 100,000 [5]. Despite its rarity, it is crucial to promptly identify precocious puberty, as it can signal underlying conditions like brain or gonadal tumours. Furthermore, early puberty can lead to complications, including impaired growth due to premature epiphyseal closure, early onset of cardiovascular diseases, and psychosocial challenges.

## Case report

A 2-year-2-month-old Malay girl was brought in by her mother, who reported that her daughter had blood in her urine. The mother noticed fresh blood staining her daughter's diapers, along with urine every time she changed them over the past two days. She did not observe any blood clots. The mother also reported that her daughter showed no signs of irritability, fever, or foul-

smelling urine. She denied any signs of injury or abuse to her daughter's genital area. Apart from a history of fever, cough, and runny nose that resolved with antibiotic treatment a week earlier, her daughter had been otherwise healthy. There were no known episodes of bleeding disorders, and her child's prenatal and developmental histories were unremarkable. The girl had no documented medical conditions, no history of hospitalisation, and was not taking any prescribed, over-the-counter, or traditional medications at the time of the visit.

An initial assessment at the health clinic revealed a non-syndromic child with normal vital signs. There were no signs of periorbital or limb oedema, abuse or neglect, or palpable abdominal masses. Examination of the genitalia showed no signs of trauma. Urinalysis at the clinic revealed erythrocytes 3+ with trace proteinuria and presence of leukocytes. Based on these findings, she was referred to a tertiary centre to evaluate for probable post-streptococcal glomerulonephritis (PSGN). Upon further questioning, it was revealed that the child had experienced two similar episodes before the current one within the past five months, and had been treated for urinary tract infection (UTI) with antibiotics on both occasions.

On the first occasion, the mother took her daughter to see a healthcare practitioner after noticing blood staining her daughter's urine-soaked diapers over the past two days. The blood was minimal and appeared only once or twice daily during diaper changes. The child appeared well during the visit, with no history of upper respiratory tract symptoms or skin lesions. Although her blood pressure was not measured, there was no documented fever. The healthcare practitioner provided a urine specimen bag and instructed the mother to return later that day with a sample collected using it. Urinalysis revealed traces of erythrocytes and leukocytes, with negative results for protein and nitrite. The child was diagnosed with a UTI and was prescribed a course of antibiotics, though no urine culture was taken beforehand. A follow-up appointment was

scheduled for one week later. By the time of the follow-up, her symptoms had resolved, and repeated urine tests showed no evidence of UTI and a negative erythrocyte count.

During the second episode, two months after the initial one, the family was on vacation when similar symptoms recurred. The mother sought treatment at a different healthcare provider and informed them of her daughter's previous UTI episode. Her daughter was also well during the visit. There was no documented fever, and she was normotensive. Urinalysis revealed the presence of erythrocytes and leukocytes. She was prescribed another course of antibiotics and advised to visit the nearest hospital or clinic for further evaluation if the symptoms persisted or recurred. Since the symptoms resolved and her daughter remained well, she did not seek further evaluation until her current episode.

Upon assessment at the tertiary centre, the paediatric team admitted her to the paediatric ward for further observation and investigations. The initial examination findings were similar to those at the health clinic, with normal vital signs and unremarkable systemic examinations. Examination of her genitalia showed no signs of external injury; the hymen was intact, with no features suggestive of abuse or foreign bodies. While in the ward, she was initially started on oral Cefuroxime to cover for a potential UTI. Her urine and blood samples were taken to investigate for both UTI and PSGN. Her repeated urinalysis, obtained using a specimen bag before antibiotic commencement, showed pale yellow urine with no evidence of UTI and negative for erythrocytes. Baseline blood investigations, including renal profile, were unremarkable, and anti-streptolysin O titre was negative. Despite daily inspections showing pale yellow urine, she continued to experience intermittent episodes of blood staining her urine-soaked diapers. A repeat urine sample obtained via catheterisation showed a similar finding to the initial one. An abdominal X-ray and ultrasound were also performed to assess for urinary tract abnormalities, stones, or renal masses. Both imaging studies revealed a normal

urinary tract system with no masses, hydronephrosis, or stones. After three days in the hospital, with a negative urine culture and ongoing episodes of blood staining her nappies, her genital area was re-examined. During this examination, minimal blood was observed coming from her vagina.

Further assessment revealed the development of sexual characteristics consistent with Tanner stage II, including both breast and pubic hair development. A significant growth spurt was observed in the child between the ages of 1 year and 2 years 3 months, during which her height increased sufficiently to cross two centile lines on the growth chart, as in Figure 1. Given these findings, her antibiotics were discontinued, and blood samples were taken for hormone studies (follicular stimulating hormone, luteinizing hormone, oestradiol, beta-human chorionic gonadotrophin, morning cortisol, thyroid function test, prolactin, insulin-like growth factor, alfa fetoprotein, and dehydroepiandrosterone sulfate (DHEA-S)). A left wrist X-ray was also carried out to assess her bone age, revealing findings consistent with that of a 3-year-old child (Figure 2). An ultrasound of the pelvis and a brain MRI were requested to investigate the underlying cause of her precocious puberty. She was referred to the paediatric endocrinologist for further evaluation and management. After one year of follow-up, and extensive investigations, she was diagnosed with central precocious puberty secondary to pituitary microadenoma. She was started on monthly GnRH analogue therapy in December 2020, a course of treatment that persists to the present day.

## Discussion

Recurrent episodes of blood mixed with urine in a diaper were evaluated as potential haematuria. Since UTI is one of the significant causes of haematuria in the paediatric population, with easily accessible urinalysis for interpretation, it is one of the most common causes of referral to the emergency department [6].

In 2000, the Paediatric Nephrology Journal published a practical primary care approach for haematuria cases in children. Based on this guideline, several steps must be taken during an investigation of possible haematuria cases. The first and most crucial step is determining whether the haematuria is gross or microscopic, as each type suggests different underlying pathologies. While the aetiology may overlap, gross haematuria often points towards a urological origin such as UTI, urethral trauma or stone; meanwhile, microscopic haematuria is commonly associated with glomerular diseases such as PSGN or IgA Nephropathy. The second step involves confirming that blood is the cause of the discoloured urine, as substances like certain foods, myoglobin, and bilirubin can also alter urine colour. Once the presence of blood in the urine is confirmed, the next step is to identify its origin—whether it is from the kidneys, upper or lower urinary tract, or a gynaecological source in girls. Determining the source of the blood is essential for guiding appropriate referrals to nephrology, urology, gynaecology, or general paediatrics for further evaluation. The final step is to determine the underlying cause of the haematuria, typically achieved after referral to a tertiary centre, as they are equipped with the necessary tools and expertise for further evaluation [7].

Urine inspection is a mandatory first step in the evaluation of haematuria. Bright red urine typically indicates lower urinary tract pathology, while tea-coloured urine suggests upper urinary tract involvement due to the oxidation of blood. A diagnosis dilemma occurred when the mother complained that bright red blood had stained her daughter's urine-soaked diaper and urinalysis showed significant erythrocytes that warrant classification into gross haematuria. However, urine inspection revealed pale yellow urine, while the other parameters showed trace leukocytes and protein. Despite being normotensive, with a prior history of URTI, it initially favours PSGN as a likely diagnosis.

However, subsequent urinalysis during hospital admission, one from a urine bag specimen and

another via catheterisation, consistently showed no signs of a UTI or presence of erythrocytes. Urine catheterisation, with 95% sensitivity and 99% specificity, is a highly reliable method for diagnosing UTIs, making it a dependable approach to rule out infection in this case [8]. Studies have also shown that contamination rates with bag urine specimens can reach up to 70% [9]. Therefore, the negative urine culture from the urine bag specimen was another reliable way to exclude UTIs.

Despite the awareness that gynaecological causes can contaminate urine samples, precocious puberty is a rare encounter. In cases like this, with an inexperienced eye coupled with an initial focus on identifying genital trauma, signs of sexual abuse and foreign bodies, it is easy to miss the secondary sexual characteristics that are right before the eyes. Failure to consider menarche when evaluating a child with gross haematuria may lead healthcare providers to undervalue identifying other critical indicators of early pubertal development during assessments, such as thelarche and growth spurt. Eventually, this can result in a missed or delayed diagnosis and management of precocious puberty.

Therefore, a thorough evaluation of the genitourinary system is crucial in cases of girls presenting with haematuria to avoid overlooking external injuries to the urethra or vagina, such as those resulting from physical or sexual abuse, or, in rare instances, to avoid missing menstruation. Early detection aids in reducing potential complications linked to PP, including the premature closure of growth plates that can result in a diminished final adult height and psychological and social difficulties arising from early physical maturation.

## **Conclusion**

This case highlights the importance of a systematic approach in evaluating children with gross haematuria. It underscores the necessity of assessing the genitourinary system to avoid

overlooking significant causes of haematuria, such as precocious puberty.

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#### **Authors' contributions**

All authors contributed equally to the concept, data collection and writing of the manuscript.

#### **Conflict of interest**

The authors declare that they have no conflict of interest.

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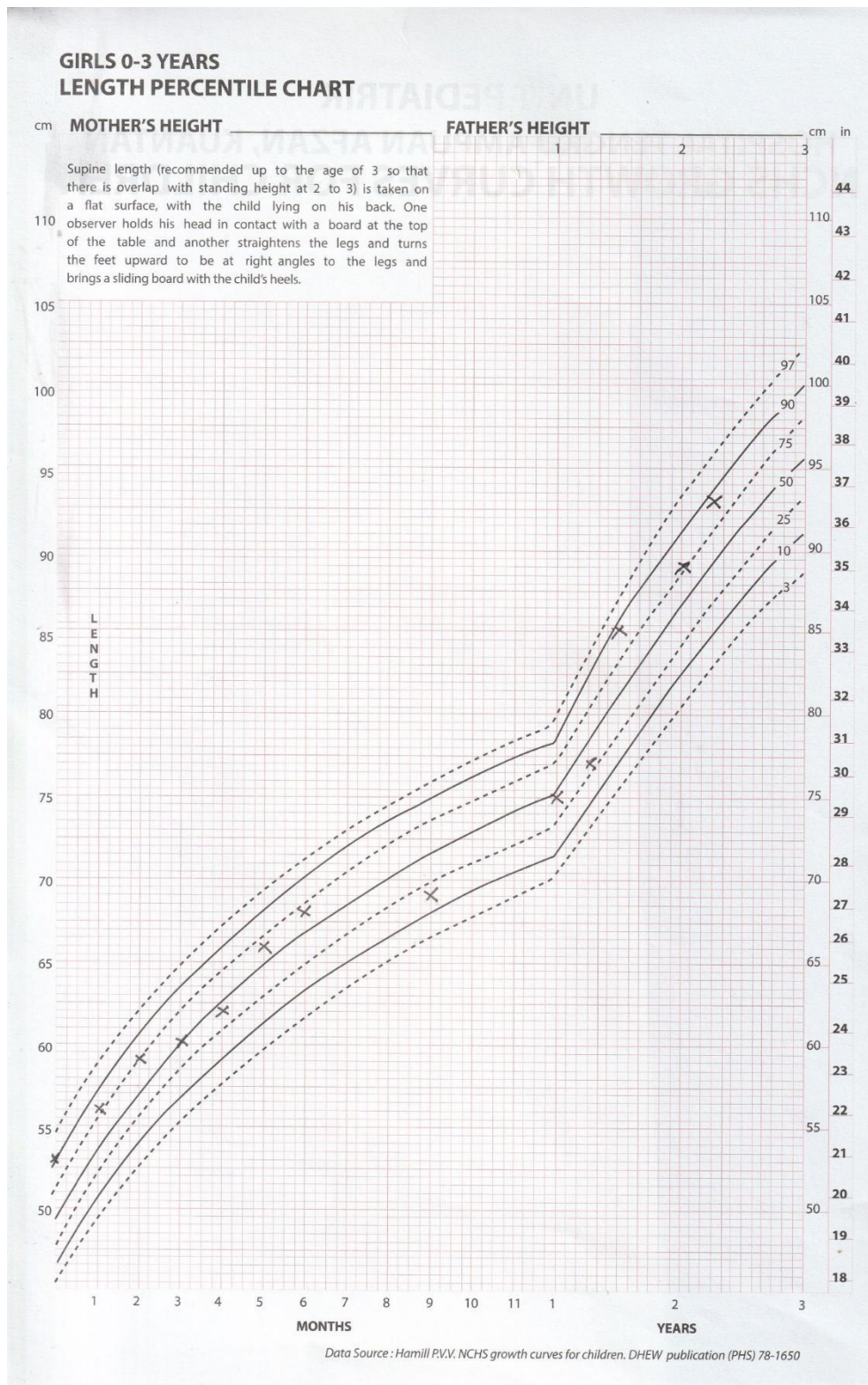


Figure 1. A significant growth spurt was observed in the child between the ages of 1-year-old and 2-year-3month-old, during which her height increased enough to cross two centile lines on the growth chart [10].



Figure 2. X-ray of the left hand and wrist showing bone age that is consistent with the skeletal age of a 3-year-old based on the Radiographic Atlas of Skeletal Development of the Hand and Wrist (Greulich and Pyle) [11]

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