Vol.59 No.1 [3/2023]

open access

Folia Medica Indonesiana

p-ISSN 2355-8398 | e-ISSN 2599-056X

Published by:
Universitas Airlangga
http://e-journal.unair.ac.id/FMI

FOLIA MEDICA INDONESIANA

p-ISSN 2355-8393, e-ISSN 2599-056X

Vol.59 No.1 [3/2023]

Published by : Universitas Airlangga

Address : Unit of Journal Consortium and Folia Medica Indonesiana
Faculty of Medicine, Universitas Airlangga
Jl. Prof. Dr. Moestopo 47 Surabaya 60131
E-mail: fmi@journal.unair.ac.id

Accredited no. 158/E/KPT/2021 by The Ministry of Education, Culture, Research, and Technology, Republic of Indonesia

FOLIA MEDICA INDONESIANA
p-ISSN 2355-8393, e-ISSN 2599-056X
Vol.59 No.1 [3/2023]

Original Research

Case Report/ Case Series

Review Article

FOLIA MEDICA INDONESIANA
p-ISSN 2355-8393, e-ISSN 2599-056X
Vol.59 No.1 [3/2023]

Original Research Report

ANXIETY DISORDER AMONG OLDER ADULTS WITH VISUAL IMPAIRMENT IN EKITI, NIGERIA: IMPLICATIONS FOR THE POTENTIAL OPPORTUNITY TO MITIGATE THE IMPACT OF THE DISABILITY AND PREVENT PSYCHOLOGICAL HARM

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ABSTRACT

Visual impairment is a significant morbidity among adults globally. It has negative impacts on work force participation and productivity, as and well as higher depression and anxiety disorder rates. In the case of older adults, vision impairment can contribute to social isolation and a greater likelihood of early entry into nursing or care homes. This hospital-based, cross-sectional study was designed to determine anxiety disorder's prevalence among the study population and which Impact of Visual Impairment (IVI) domain is most correlated with anxiety disorder. The study was carried out at the Ophthalmology Clinic of the Ekiti State University Teaching Hospital, Ado Ekiti, Nigera. Questionnaires were used to collect information from 115 respondents. Information sought included sociodemographic information, anxiety status, and visual impairment's impact on daily functions. The Hospital Anxiety and Depression Scale (HADS) was used to assess anxiety disorder, while the IVI scale was used to assess the impact of visual impairment. The IVI scale is an instrument used for determining the impact of visual impairment under three domains: (reading and accessing information, mobility and independence, and emotional well-being). Anxiety disorder was detected in 10.4% of all the respondents, and the IVI's mobility and independence domain of IVI was most strongly correlated with anxiety disorder.

Keywords: Older adults; Impact of Visual Impairment; anxiety disorder; psychological harm; mental disorder

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Article history
●Submitted 16 Dec 2022 ● Revised 7 Jan 2023 ● Accepted 22 Feb 2023 ● Published 10 Mar 2023

How to cite: Aina FO, Ajayi IA, Omotoye OJ, et al (2023). Anxiety disorder among older adults with visual impairment in Ekiti, Nigeria: Implications for the potential opportunity to mitigate the impact of the disability and prevent psychological harm. Folia Medica Indonesiana, 59 (1), 1-7. https://doi.org/10.20473/fmi.v59i1.42741

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. Anxiety is an important morbidity among visually impaired older adults.
2. There is potential for stakeholders to specifically channel their interventions towards particular IVI domains in order to reduce anxiety prevalence.

INTRODUCTION

Globally, at least 2.2 billion people have a near or distant vision impairment. In about half of these cases, a visual impairment could still be prevented or has yet to be addressed (World Health Organization 2022). Approximately 26.3 million people in the African region have a form of visual impairment. Of these, 20.4 million have low vision and 5.9 million are estimated to be blind (World Health Organization 2021). The 2019 definition of visual impairment by the World Health Organization (WHO), as quoted by Naipal & Rampersad (2018), is a disorder that occurs in a person who has impairment of visual functioning even after treatment and/ or standard refractive

correction, a visualacuity (VA) of less than 6/18 to light perception, or visual field of less than 10 degrees from the point of fixation, but who uses or is potentially able to usevision for the planning or execution of tasks.

According to the WHO, visual impairment severely impacts the quality of life among adult populations. Adults with visual impairment often have lower rates of work force participation and productivity, as well as higher rates of depression and anxiety disorder. Visual impairment in older adults can contribute to social isolation, difficulty walking, a higher risk of falls and fractures, and a greater likelihood of early admission tonursing homes or care facilities (World Health Organization 2022). The eleventh revision of the International Classification of Diseases (ICD-11) classified distance visual impairment into four categories: mild (VA worse than 6/12 to 6/18), moderate (VA worse than 6/18 to 6/60), severe (VA worse than 6/60 to 3/60), and blindness (VA worsethan 3/60) (World Health Organization 2022).

Malhotra et al. (2018) found a visual impairment prevalence of 24.5% among older adults in a rural community in northern India. Another study reported that glaucoma was most commonly found in people aged 50-64 years (Yolanda et al. 2021, Praba et al. 2021). Unfortunately, treatment of late-stage glaucoma through medication, surgery, and combination therapies did not result in significant improvement (Pratista et al. 2022). In Nigeria, the National Blindness and Visual Impairment Survey conducted in 2005-2007 reported that 4.2% of people aged >40 years were blind and 11.5% had moderate to severe visual impairment (Gascoyne et al. 2022).

The Hospital Anxiety and Depression Scale-Anxiety Subscale (HADS-A) has been used to demonstrate a link between visual impairment and anxiety disorder in older adults, focusing on the prevalence of anxiety disorder and the related risk factors (Kempen & Zijlstra2014, Heesterbeek et al. 2017). Impaired vision is associated with difficulty in daily tasks, and addressing this is the primary goal of vision rehabilitation. Although the Impact of Visual Impairment (IVI) questionnaire was designed to assess the rehabilitation needs of visually impaired persons, the results of its use can guide clinicians in taking preventative measures to either delay or prevent morbidities associated with visual impairment.

The objectives of this research were, firstly, to determine the prevalence of anxiety disorder among the study population and, secondly, to identify which domain of the IVI is more correlated with anxiety disorder. This second objective was significant due to the scarcity of literature on the topic. It would help clinicians and policymakers mitigate the impact of visual impairment by targeting a particular domain of the IVI for intervention and potentially lowering the prevalence of anxiety disorder.

MATERIALS AND METHODS

This cross-sectional study was carried out at the Ophthalmology Clinic of Ekiti State University Teaching Hospital, Ado Ekiti, Nigeria, over a period of three months from September to November 2022. All consenting older adults aged 60 and above who presented at the clinic and consented to participate in the study were consecutively recruited on a daily basis.

Information sought from the respondents included the socio-demographic characteristics and duration of visual impairment. The Impact of Vision Impairment (IVI) questionnaire was used to assess the impact of visual impairment, while the Hospital Anxiety and Depression Scale-Anxiety Subscale (HADS-A) was used to assess anxiety. The respondents were classified into normal, borderline, and abnormal groups. Their visual acuity was evaluated by an ophthalmologist and an ophthalmic nurse who had been trained on the study's protocol and procedures. The IVI questionnaire consists of 28 items that measure the impact of visual impairment on participation in daily activities across three domains, i.e., reading and accessing information, mobility and independence, and emotional well-being. The IVI has been used and validated by researchers in various settings (Finger et al. 2014, Ratanasukon et al. 2016). The HADS is a valid method for assessing anxiety disorder and depression among older adults through a 14-item questionnaire (Djukanovic et al. 2017). The scale consists of two 7-item subscales for symptoms of anxiety disorder and depression. Each item is coded from 0 to 3. The respondents were classified as normal, borderline, or abnormal based on their HADS-A scores. A score of 0 to 7 indicated no anxiety symptoms, 8 to 10 indicated moderate and doubtful symptoms, and ≥11 indicated a confirmed case of anxiety.

IBM SPSS statistic for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) was utilized as appropriate to perform analyses of the frequency distribution, Chi-square, Fisher's exact, and correlation. For statistical purposes, the IVI questionnaire scores were used to divide the respondents into two categories: those who scored <50% of the maximum score for the overall and each domain were classified as having a moderate impact, while those who scored ≥50% were

classified as having a severe impact. In addition, the visual impairment was graded as moderate, severe, or blindness. The "mild impairment" class in the International Classification of Diseases was fused with "no impairment".

RESULTS

A total of 115 respondents participated in this study. The age of the respondents was between 60 and 102 years, with a mean age of 71.4±8.30 years. The majority of the respondents (80%) were between the ages of 60 and 79, with a female preponderance (56.6%). About half (50.4%) of the respondents had been suffering from visual impairment for one to five years. Most respondents (73.9%) were having moderate impairment, while (25.2%) were blind (Table 1).

Table 1. Socio-demographic characteristics of the respondents.

Age distribution (n=115)		
Age (year)	Frequency	Percentage (%)
60–69	57	49.6
70–79	35	30.4
80–89	18	15.6
90–99	4	3.5
≥100	1	0.9
Total	115	100.0
Sex distribution (n=113)		
Male	49	43.4
Female	64	56.6
Total	113	100.0
Marital status (n=112)		
Married	71	63.4
Divorced	6	5.4
Widow	35	31.2
Total	112	100.0
Duration of illness (n=113)		
Duration (year)	Frequency	Percentage (%)
<1	10	8.9
1–5	57	50.4
6–10	33	29.2
11–15	8	7.1
16–20	5	4.4
Total	113	100.0
Degree of vision impairment (n=115)		
Degree	Frequency	Percentage (%)
Moderate	85	73.9
Severe	1	0.9
Blindness	29	25.2
Total	115	100.0

The prevalence of anxiety disorder among the respondents was 10.4%. There was no statistically significant relationship between the respondents' socio-demographic characteristics or duration of illness and anxiety disorder (Table 2).

Table 2. Relationship between socio-demographic characteristics and anxiety disorder.

	N	B	AN	Fisher's exact test (2-sided)
Age (year)				
60–69	50	3	4	
70–79	28	0	7	
80–89	16	2	0	0.039
90–99	2	1	1	
≥100	1	0	0	
Sex				
Male	43	1	5	0.442
Female	53	5	6	
Religion				
Christianity	91	5	10	0.472
Islam	3	0	1	
Marital status				
Married	61	4	6	
Divorced	5	0	1	0.818
Widow	30	1	4	
Duration (year)				
<1	7	1	2	
1–5	50	1	6	
6–10	27	3	3	0.428
11–15	6	1	1	
16–20	5	0	0	

N=normal
B=borderline
AN=abnormal

Table 3. Relationship between visual impairment and anxiety disorder.

Impairment	N	B	AN	Fisher's exact test (2-sided)
Moderate	76	3	6	
Severe	0	1	0	0. 007
Blindness	21	2	6	

N=normal
B=borderline
AN=abnormal

Table 4. Association between the overall impact of visual impairment and anxiety disorder.

	N	B	AN	Fisher's exact test (2-sided)
Moderate	85	2	80	0. 000
Severe	12	4	4	

N=normal
B=borderline
AN=abnormal

Table 3 demonstrates that there was a statistically significant association between visual impairment

and anxiety disorder (two-sided Fisher's exact=0.007). There was also a statistically significant association between the level of overall impact of visual impairment and anxiety disorder (two-sided Fisher's exact=0.000) (Table 4).

Table 5. Correlation between the three domains of the IVI and anxiety disorder.

Domain	Corr. Coeff	P-value
Reading and accessing information	0.246	0.008
Emotional well-being	0.463	0.000
Mobility and independence	0.663	0.000*

*Significant

Table 5 summarizes the correlations between anxiety disorder and the three domains of the IVI, i.e., reading and assessing information (r=0.246, P=0.008), emotional well-being (r=0.463, P=0.000), and mobility and independence (r=0.663, P=0.000).

DISCUSSION

This study discovered a 10.4% prevalence of anxiety disorder in the studied population. This is consistent with earlier findings that showed the prevalence of anxiety disorder among people with age-related macular degeneration ranged between 9.6% and 30% (Dawson et al. 2014, Cimarolli et al. 2015). The frequent consumption of antipsychotics and antidepressants among older adults might indicate that physical comorbidities influence mental disorders in this age group (Zhong et al. 2018, Dong et al. 2019). van der Aa et al. (2015) discovered a prevalence of 7.5% among visually impaired older adults using the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria. A study conducted by Gascoyne et al. (2022) in Nigeria reported that the prevalences of anxiety among male older adults were 12.7% and 12.4% among females. Another study, also conducted in Nigeria and employed the same instrument, found that the prevalences of anxiety among male and female participants aged 18 years and older with glaucoma andcataract were 35.5% and 21.8%, respectively (Okudo et al. 2021). Ulhaq et al. (2022) observed a 19.0% pooled prevalence of anxiety disorders among ophthalmic disease patients. The female preponderance in this study is also in agreement with previous studies (Aina et al. 2018, Fadare et al. 2021).

Previous findings support the established relationship between visual impairment, including its severity, and anxiety disorder. Demmin & Silverstein (2020) found an association between visual impairment and anxiety disorder. A study by Gascoyne et al. (2022) in Kogi State, Nigeria, reported that severe visual impairment was more associated with anxiety disorder. Similarly, Assi et al. (2021) found that worse visual impairment was linked to lower quality of life. Various quality studies have provided findings on the associations between visual impairment and many factors, such as the well-being and functioning of the patients.

The demonstration of a strong positive correlation between the mobility and independence domain of the IVI and anxiety disorder was novel in this study, as there was a scarcity of literature on this topic. The findings of this study might be related to the psychopathology of anxiety disorder, which has been linked to worry and fear. The IVI questionnaire posed aquestion on the participants' concerns or worries about several factors due to their eyesight. The Diagnostic and Statistical Manual of Mental Disorders V (DSM-V) Workshop on Anxiety Disorder proposed four worry behaviors to be included in the diagnosis criteria of generalized anxiety disorder (GAD) (Brown & Tung 2018). Worry behaviors contributed modestly to the diagnostic classification of GAD and may be salient to treatment planning, treatment response, and the natural course of the disorder. This study therefore corroborated the relationship between worry behaviors and anxiety.

Most questions under the mobility and independence domain of the IVI were about the potentials that triggered a sense of fear or worriness in the respondents. Fear experienced by older adults with a visual impairment included fear of moving around on an unknown territory, fear of falling, and fear of staying in a particular place or situation (Binder et al. 2020). Apart from the aforementioned kinds of fear, the mobility and independence domain of the IVI also included fear about safety inside and outside of the home, fear of going down steps or stairs, and fear of soiling or breaking things. Although the fear and anxiety conundrum has been a subject of debate, the two can be used interchangeably, which appears to be the position endorsed by the ICD-11 through the designation of the "anxiety or fear-related disorder" diagnostic group (World Health Organization 2019, Starcevic et al. 2020). It further shows that fear is closely linked to anxiety.

In terms of outcome, an association has been established between visual impairment and anxiety. Senra et al (2022) found that a higher degree of vision loss was associated with a poorer quality of life, and this relationship was stronger when the level of anxiety was high. Individuals suffering from anxiety have demonstrated significant impairment in global, social, occupational, and physical domains. Previous

research also emphasized the detrimental impact of anxiety on numerous functional domains of life, with a negative influence on quality of life (McKnight et al. 2016, Wilmer et al. 2021). Therefore, anxiety disorder deserves serious attention in the care of older adults with visual impairment.

Strength and limitations

This study's novelty is expected to contribute to the scarce literature on visual impairment as morbidity in older adults and its correlation to anxiety disorder. Stakeholders may use the findings of this study to direct interventions toward the mobility and independence domain of the IVI in order to minimize anxiety prevalence among older adults with visual impairment. However, because it was a hospital-based study, generalization may be a challenge because the findings may not be representative of the general population. Despite this limitation, the potential opportunity it provides in terms of knowledge, practice, and policy justifies its merit.

CONCLUSION

This work goes beyond demonstrating that visual impairment is associated with anxiety. As reported, it goes further to show which IVI domain correlates most with anxiety disorder. This finding has implications for preventing anxiety among visually impaired older adults. It has been suggested that studies should involve evaluations of worry behaviours' role in generalized anxiety disorders maintenance and treatment. This study's results will also improve services provided by clinicians and other stakeholders involved in rehabilitation. Clinicians can reduce worry and anxiety disorders among this group through counselling, which can be reinforced further during rehabilitation. The role of policy makers includes but is not limited to providing mobility devices and creating special passages for the visually impaired in public places.

Acknowledgment

The authors acknowledge the cooperation of the staff at the Ophthalmology Clinic of Ekiti State University Teaching Hospital, Ado Ekiti, Nigeria, during the research period.

Conflict of interest

None.

Ethical consideration

This study was approved by the Ethics and Research Committee of Ekiti State University Teaching Hospital, Ado Ekiti, Nigeria, with a reference no. EKSUTH/A67/2022/09/003 on 01/09/2022.

Funding disclosure

None.

Author contribution

AFO, AIA, ATA, and FJO contributed to the literature conceptualization and review. AFO, OOJ, ATA, and PP contributed to the study design and methodology. AFO, AIA, OOJ, and AKO contributed to the data collection. AFO, DMU, FJO, and AKO contributed to the data analysis. AFO, ATA, FJO, DMU, AKO, and PP contributed to the discussion of this study's findings. All authors gave final authorization of the completed work.

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Original Research Report

SCREEN TIME AND DRY EYE DISEASE DURING DISTANCE LEARNING AMONG THE CLASS OF 2019 MEDICAL STUDENTS AT A UNIVERSITY IN JAKARTA, INDONESIA

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ABSTRACT

Dry eye disease (DED), also known as dry-eye syndrome, is commonly caused by a lack of tears or excessive evaporation. It affects 334 million people worldwide. According to global epidemiological research, the DED prevalence in adults and the elderly is roughly 5-50%. Research has revealed that DED prevalence and epidemiology are more typically connected to aging. However, extended exposure to digital screens is an external DED risk factor to be aware of, especially in this digital technology era and amidst the COVID-19 pandemic. These two situations are expected to increase the number of DED sufferers in the younger age groups, such as elementary, high school, and university students. The ongoing COVID-19 pandemic has compelled a shift in learning and teaching methods in order for education to proceed. However, the digitization era and the COVID-19 pandemic can double the DED risk, particularly among medical students. This study's objective was to determine how remote learning affected DED prevalence among the class of 2019 medical students at Universitas Tarumanagara, Jakarta, Indonesia, in the COVID-19 pandemic. Data were collected from 144 respondents using a Google Forms questionnaire. According to the statistical test results, there was a 2.4-fold increase in screen time during the COVID-19 pandemic compared to the pre-pandemic period. The results demonstrated a significant relationship between screen time and DED among the class of 2019 medical students at Universitas Tarumanagara. These findings are predicted to be of interest to academics and students, as their frequent engagement with computer displays and smartphones will continue to increase due to technological advancements. Furthermore, researchers may use the findings as a reference in conducting DED-related research.

Keywords: Dry eye disease (DED); technology; COVID-19; healthy eyes; human and health

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Article history
●Submitted 17 Dec 2022 ● Received 27 Jan 2023 ● Accepted 22 Feb 2023 ● Published 10 Mar 2023

How to cite: Bernadette K & Rasyid M (2023). Screen time and dry eye disease during distance learning among the class of2019 medical students at a university in Jakarta, Indonesia. Folia Medica Indonesiana. 59 (1), 8-13. https://doi.org/10.20473/fmi.v59.i1.38737

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. There is a correlation between screen time and dry eye disease among medical students due to distance learning during the pandemic.
2. This study's findings can lead researchers to conduct further studies to investigate and prevent DED.

INTRODUCTION

Dry eye disease (DED), often known as dry eye syndrome, is a condition caused by a lack of fluid in the eyes or excessive evaporation. This condition affects around 334 million people around the world (Favero et al. 2021, Agarwal et al. 2021). According to global epidemiological research, the DED prevalence among adults and the elderly ranges between 5% and 50% (Stapleton et al. 2017). In the age of cutting-edge digital technology and the COVID-19 pandemic, prolonged exposure to digital screens is a risk factor that must be monitored in order to prevent DED. Internet connectivity, cellphones, tablets, and other similar technology have made life more convenient (Mehra & Galor 2020). The convenience offered by technology leads users to spend more time glued to their digital devices. It increases the DED prevalence in the younger generation, such as

school-aged children and college students (Yazdani et al. 2019, Kawashima et al. 2020, Naderi et al. 2020).

The present COVID-19 pandemic has forced changes to teaching and learning approaches in order to maintain the continuity of the educational process (Cartes et al. 2022). Face-to-face learning has been substituted for distance learning or fully online learning, which has its own set of mental and physical health consequences, such as digital eye strain from prolonged computer and smartphone use (Sheppard & Wolffsohn 2018, Khariri et al. 2022). A study found that studying in front of a computer screen for more than three hours increases the risk of developing DED (Thun-Hohenstein et al. 2021). Medical students are at a higher risk of exposure due to the heavy workload from online lectures, discussions, case discussions, paper writing, journal searches, and e-book learning (Albert 2020, Cartes et al. 2022). The digitization era, as well as the ongoing COVID-19 situation, can increase the double risk of DED, particularly among medical students (Usgaonkar et al. 2021, Ardyan et al. 2021). This study examined the association between distance learning and the risk of DED among students at the Faculty of Medicine, Universitas Tarumanagara, Jakarta, Indonesia. The findings of this study may be useful for academics and students, whose use of computer screens and smartphones continues to grow as technology advances. Future clinical investigations evaluating the relationship between DED and screen time are expected to support the findings of this study.

MATERIALS AND METHODS

This study employed a cross-sectional study design. A two-week online survey through Google Forms was provided, which included demographics, health conditions, medical history, the Ocular Surface Disease Index (OSDI), and screen time before and during the pandemic. The participants in this study were the class of 2019 students at the Faculty of Medicine, Universitas Tarumanagara, Jakarta, Indonesia. The sample size for this study was determined using a single sample proportion formula, and 123 students were necessary. Data were acquired from the students who were engaged in distance learning activities and willing to participate in this research by filling out the questionnaires. Exclusion criteria for this study were students from the class of 2019 at the Faculty of Medicine, Universitas Tarumanagara, who had been diagnosed with autoimmune diseases (such as Sjogren's syndrome, rheumatoid arthritis, sarcoidosis, Bell's palsy, diabetes, and thyroid disorders) or who were taking antihistamines, beta-blockers, decongestants, diuretics, selective serotonin reuptake inhibitors (SSRIs), anxiolytics, tricyclic antidepressants, antipsychotics, and oral isotretinoin for more than three months.

The change in screen time for distance learning was the independent variable of this study, while DED was the dependent variable. The Shapiro-Wilk test was used to assess data normality with a significance value (p) of 0.05, and the Chi-square test was used to determine independence or correlation (Razali & Wah 2011, McHugh 2013). The class of 2019 students of the Faculty of Medicine, Universitas Tarumanagara, consented to the publication of this study.

RESULTS

In the span of two weeks, 154 medical students completed the questionnaires and agreed to be research respondents. At the data cleaning stage, there were 10 students who had regularly consumed drugs in the previous three months that could have interfered with the objectivity of the study, including 7 students (70%) consuming anti-histamines, a student (10%) consuming anti-depressants, a student (10%) consuming anti-histamines and anti-psychotics, and a student (10%) consuming anti-depressants and antipsychotics. After excluding the ten students, the number of participants who met the inclusion criteria was 144, with 70.8% being females (n=102) and 29.2% being males (n=42).

Table 1. Characteristics of respondents according to the completed questionnaires.

Characteristics	Frequency (n)	%
Sex		
Male	42	29.2
Female	102	70.8
Screen time before the pandemic		
<1 h	1	0.7
1-4 h	36	25.0
4-8 h	68	47.2
8-12 h	27	18.8
>12 h	12	8.3
Screen time after the pandemic		
<1 h	1	0.7
1-4 h	10	6.9
4-8 h	33	22.9
8-12 h	71	49.3
>12 h	29	20.1

As seen in Table 1, there had been an increase in the amount of screen time during the COVID-19 pandemic. Prior to the pandemic, the majority of respondents (47.2%) spent 4-8 hours in front of a screen, with the second majority (25%) spending 1-4 hours. During the pandemic, the majority of the

respondents increased their screen time from 4-8 hours (22.9%) to 8-12 hours (49.3%). The proportion of respondents with 8-12 hours of screen time surged 2.6 times, while the proportion with more than 12 hours of screen time increased 2.4 times. The distribution of less than an hour of screen time per day was only 0.7%, with a minimum ratio to other screen time groups.

Table 2. Categorization of dry eye severity among the respondents based on the screen time groups.

Characteristics	Category of dry eye severity n (%)				Total
	Normal	Mild	Moderate	Severe	
Sex					
Male	35 (83.3)	4 (9.5)	2 (4.8)	1 (2.4)	42
Female	66 (64.7)	29 (28.4)	5 (49)	2 (2.0)	102
Screen time before the pandemic					
1-4 h	29 (78.3)	7 (19.0)	0 (0.0)	1 (2.7)	37
4-8 h	51 (75.0)	11 (16.2)	4 (5.9)	2 (2.9)	68
8-12 h	16 (59.3)	9 (33.3)	2 (7.4)	0 (0.0)	27
>12 h	5 (41.7)	6 (50.0)	1 (8.3)	0 (0.0)	12
Screen time during the pandemic					
1-4 h	10 (91.0)	1 (9.0)	0 (0.0)	0 (0.0)	11
4-8 h	26 (78.8)	5 (15.2)	1 (3.0)	1 (3.0)	33
8-12 h	50 (70.4)	15 (21.1)	5 (7.0)	1 (1.4)	71
>12 h	15 (51.7)	12 (41.4)	1 (3.4)	1 (3.4)	29

Table 1 summarizes the characteristics of the respondents, while Table 2 categorizes the severity of dry eye based on the reported symptoms. The female respondents were more likely experience dry eyes (35.3%) than male respondents (16.7%). Furthermore, both before and during the COVID-19 pandemic, there was a propensity for increasing dry eye symptoms, which worsened with increased screen time.

Table 3. Data normality test of the OSDI scores based on the screentime groups.

Category	Screen time			
	Before the pandemic		After the pandemic	
	p-value	Conclusion	p-value	Conclusion
1-4 h	0.004	Abnormal	0.000	Abnormal
4-8 h	0.000	Abnormal	0.000	Abnormal
8-12 h	0.000	Abnormal	0.017	Abnormal
>12 h	0.048	Abnormal	0.050	Abnormal

p: Significance

The first analysis of the Ocular Surface Disease Index (OSDI) was performed to compare dry eye disease over various screen time groups. The OSDI score comparison started with a data normality test in each screen time. The Shapiro-Wilk normality test (observation of <30 per group) revealed an abnormal distribution ($p<0.05$), as seen in Table 3. Finally, a comparative analysis using the Kruskal-Wallis test was performed and described in Table 4. There were significant differences in the mean and median OSDI scores ($p<0.05$) among the four screen time groups during the COVID-19 pandemic (Table 3). Conversely, there was no significant difference in the mean and median OSDI scores for the four screen time groups prior to the pandemic ($p>0.05$).

Table 4. Comparative test of the OSDI scores based on the screentime groups.

Category	Screen time			
	Before the pandemic*		During the pandemic**	
	Average±SD	Median	Average±SD	Median
1-4 h	7.3±8.4	5.0	4.3±5.1	3.0
4-8 h	9.0±8.1	7.0	7.6±8.8	6.0
8-12 h	10.4±8.2	7.0	9.7±8.8	8.0
>12 h	12.3±7.5	14.0	11.2±8.6	9.0

SD: Standard deviation; *p=0.11; **p=0.02;
p: Significance; p=0.05

The second analysis included a Chi-square test of independence with categorical variables and a correlation test, as depicted in Table 5. This analysis was performed by reclassifying normal and dry eye conditions. The screen times were classified as <8 hours, 8-12 hours, and >12 hours groups due to some 0 observations in the other screen time group. Furthermore, the results of the correlation test revealed that screen time had a significant effect on the incidence of dry eye before and during the pandemic.

Table 5. Correlation of screen time with the respondents' eye conditions.

Screen time	Eye conditions based on the OSDI scores		p
	Normal n (%)	Dry n (%)	
Before the pandemic			
<8 h	80 (76.1)	25 (23.9)	
8-12 h	16 (59.2)	11 (40.8)	0.04
>12 h	5 (53.4)	7 (46.4)	
During the pandemic			
<8 h	36 (82.0)	8 (18.0)	
8-12 h	20 (48.7)	21 (51.3)	0.02
>12 h	15 (51.7)	14 (48.3)	

DISCUSSION

A study by Sheppard & Wolffsohn (2018) found that sitting in front of a computer screen for more than three hours raised the risk of DED considerably. The findings are also consistent with a study conducted by Cartes et al. (2022) on the

association between screen time and dry eye symptoms among 1,450 university students during the COVID-19 epidemic. They also mentioned a considerable rise in average screen time prior to and after the pandemic (9.8 hours vs. 15.9 hours, respectively). Most countries, including Indonesia, implemented widescale isolation, quarantine, and social restrictions when the World Health Organization (WHO) declared the COVID-19 pandemic (Bulut & Kato 2020, Adam et al. 2021). When lockdown policies were implemented in many countries, increased screen time and a lack of outdoor activity led to an increase in myopia prevalence (Savitri et al. 2022).

To boost productivity, several methods of working and studying at home have been proposed, including fully online or remote learning, lecturing, and working (Bahkir & Grandee 2020, Sievertsen & Burgess 2020, Adam et al. 2021). The implementation of these learning methods mostly involves video display terminals, which require extensive close-up work and may predispose to DED among workers and students (Hasanah et al. 2017, Bahkir & Grandee 2020, Loebis et al. 2021). Continuously using digital terminals for long periods of time, especially without intermittent breaks, has been demonstrated to impair eye health and induce discomfort by disrupting tear stability and creating mild inflammation (Latkany et al. 2014). Furthermore, excessive screen time can result in accommodative dysfunction and divergence responses (Yuan et al. 2021).

In this study, the prevalence of dry eyes was 29.8%. This number was quite low when compared to recent studies evaluating the OSDI scores. Condori et al. (2021) discovered that 70.6% of university students in Peru had symptomatic dry eyes. In comparison, Alkabbani et al. (2021) discovered a 62.6% prevalence of dry eyes among university students in Dubai. Another study on college students showed a high prevalence of computer vision syndrome, but it could improve with the administration of sodium hyaluronate (Zulkarnain et al. 2022). However, because DED is a complex disorder that includes environmental factors, differences in climate and humidity in each research region may have an effect (Madden et al. 2013, Tesón et al. 2013).

The results of this study suggested that the female respondents had a higher prevalence of dry eyes than the male respondents. Cartes et al (2022) and Supiyaphun et al (2021) also similarly argued that female students more typically experienced dry eye symptoms ($p<0.05$). Several female-predominant factors, such as contact lens use, were identified in addition to hormonal factors that played a role in the development of dry eyes. The comparison and correlation test results demonstrated that screen time was related to the occurrence of dry eye symptoms, particularly during the COVID-19 pandemic ($p<0.05$).

These findings are consistent with the majority of studies on the association between screen time and dry eyes among college students. Cartes et al. (2022), Condori et al. 2021), Alkabbani et al. (2021), and Supiyaphun et al. (2021) were some of the researchers who discovered that screen time was related to the occurrence and severity of dry eyes. Tear hyperosmolarity, also known as tear filminstability, significantly contributes to DED. A reduced blink reflex has been recognized as a risk factor for tear hyperosmolarity in computer users (Talens-Estarelles et al. 2021). Sánchez-Valerio et al. (2020) emphasized that electronic devices do not cause organic damage but rather influence the emergence of exhaustion or asthenic symptoms when misused or utilized for an extended period of time. Prolonged use of electronic devices can also cause a decrease in blinking, lacrimal gland changes, sensorineural damage, and a hindered distribution of adequate tear film lipid layers. DED, ocular surface injury, and eyelid disruption may emerge as a resultof these conditions.

Strength and limitations

This research could be beneficial to academics and students since it provides an observation on the occurence of DED due to prolonged electronic deviced during the pandemic. However, the sample size of this study was rather small compared to other studies that investigated a larger population. Future research can be conducted based on the findings of this study in order to investigate DED among college students on a larger scale and to prevent its prevalence.

CONCLUSION

During the pandemic, there was a higher propensity for medical students to spend more time in front of screens. Dry eye disease (DED) became prevalent among the class of 2019 students at the Faculty of Medicine, Universitas Tarumanagara, Jakarta, Indonesia, due to prolonged electronic device usage during distance learning. Screen time was found to be related to the occurrence of dry eyes among the students.

Acknowledgment

The authors would like to thank the Research and Community Service Unit and class of 2019 students at the Faculty of Medicine, Universitas Tarumanagara, Jakarta, Indonesia.

Conflict of interest

None.

Ethical consideration

The study was ethically approved by the Research and Community Service Unit of the Faculty of Medicine, Universitas Tarumanagara, Jakarta, Indonesia, with the approval letter no. 170/KEPK/UPPM/FK UNTAR/XI/2021 on 29/11/2021.

Funding disclosure

None.

Author contribution

KB conceptualized the study and methodology, conducted the investigation and formal analysis, and drafted the initial manuscript. MR validated and supervised the study, reviewed and edited the manuscript, curated the data, provided resources, and managed the project administration.

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Original Research Report

NUTRITIONAL KNOWLEDGE AND BODY MASS INDEX AMONG STUDENTS AT NOVENA UNIVERSITY, OGUME, NIGERIA

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ABSTRACT

Body mass index (BMI), which is calculated using height and weight, is a rough indicator of body fat. This study aimed to investigate whether there is a significant correlation between nutritional knowledge and BMI among students at Novena University, Ogume, Nigeria. This study was done using a cross-sectional survey. Interviews were conducted with 50 participants from the sample, whose nutritional knowledge and weight status were assessed. The results showed that the students' BMI ranged from 15 to 39, with a mean and standard deviation of 23.93±5.46 cm. There was a positive correlation between the students' nutritional knowledge and their BMI. In conclusion, the majority of Novena University students are knowledgeable about obesity, which likely accounts for their low obesity prevalence rate.

Keywords: Nutritional value; anthropometry; body mass index; Nigeria

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Article history
●Submitted 30 Nov 2022 ● Revised 21 Jan 2023 ● Accepted 15 Feb 2023 ● Published 10 Mar 2023

How to cite: Andrew UO, Godswill OO, Mamerhi ET, et al (2023). Nutritional knowledge and body mass index among students at Novena University, Ogume, Nigeria. Folia Medica Indonesiana, 59 (1), 14-19. https://doi.org/10.20473/fmi.v59i1.39977

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. Interviews were conducted on the correlation between nutritional knowledge and BMI among students at Novena University, Ogume, Nigeria.
2. Most of the students had adequate nutritional knowledge and a low obesity prevalence rate.

INTRODUCTION

Obesity has emerged as the most common dietary problem around the globe, and Nigeria is no exception. It is becoming more prevalent among Nigerians. Being overweight or obese shortens life expectancy, particularly in younger age groups, and increases the risk of various diseases and health conditions (e.g., hypertension, gallstones, coronary heart disease, and diabetes) (Munir et al. 2016, Nugroho & Martini 2020, Putri et al. 2022, Lysandra et al. 2022). Several of the underlying causes include individual behaviors, environmental factors, heredity, and being overweight due to a long-term energy imbalance. A healthy weight can be maintained by eating a variety of foods, such as plenty of fruits, vegetables, and whole grains (Pereira et al. 2012, Zhou et al. 2017, World Health Organization 2022).

Body mass index (BMI) can be used to estimate a person's body fat by measuring their height and weight. It is calculated by dividing the body mass (kg) by the squared body height (m) (Valmórbida et al. 2017, Hammond et al. 2022). The World Health Organization (2000), as cited by Hruby & Hu (2015), considered BMI as the most widely employed indicator of adult nutritional status and the only parameter with comparable data that are typically available. People who have poor nutritional knowledge and attitudes frequently engage in inappropriate eating behaviors; therefore,

implementing educational intervention programs could improve their nutritional awareness (Ilori & Sanusi 2022, De Craemer et al. 2022). Hence, this study investigated whether there was a significant correlation between nutritional knowledge and BMI among students at Novena University, Ogume, Nigeria.

MATERIALS AND METHODS

This study employed a multistage cluster sampling technique to collect data from 50 students, consisting of 27 males and 23 females. First-year, second-year, third-year, and fourth-year students participated in this study. The inclusion criteria were healthy subjects with no physical deformities. Students enrolled in part-time, post-diploma, and post-graduate programs at the institution were excluded from this study. The students' body weights were assessed using Hammond's formula for calculating body weight (Peterson et al. 2016). The classification of body mass according to the BMI formula is as follows: 18.5 indicates underweight, 18.5-24.9 indicates normal weight, 25.0-29.9 indicates overweight, 30.0-34.9 indicates class I obesity, 35.0-39.9 indicates class II obesity, and ≥40 indicates class III extreme obesity.

BMI was utilized because it is a recognized indicator of body weight status in anthropometry (Widjaja et al. 2019). The weight was determined using a calibrated electronic platform scale with an accuracy closest to 100 grams. On a long platform, the height was determined using a measuring tape. The participants stood with their heels together, arms to the side, legs straight, shoulders relaxed, and heads in the Frankfort horizontal plane as instructed by the researchers. The interview questions were developed around the sociodemographic information and nutritional awareness of the participants, which may include the most and least frequently consumed food groups, the servings of each food group, and nutritional knowledge on foods with high and low fat, sugar, and fiber contents. The questions about nutritional knowledge should be answered with “correct”, “incorrect”, and “I do not know”. The data were analyzed using the IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA). Descriptive statistics were generated as well as the Pearson’s correlation coefficient.

RESULTS

Among the respondents in this study, 27 (54%) were male students and 23 (46) were female students (Table 1). The respondents were selected from different levels of study, i.e., 13 (26%) first-year students, 11 (22%) second-year students, 10 (20%) third-year students, and 16 (32%) final-year students. The majority of the students resided in the university hostel and the remainder lived elsewhere.

Table 2 presents the eating practices and nutritional knowledge of the students. Half of the students (50%) ate twice a day, 15 (30%) ate three times a day, and 9 (18%) ate four times a day. Only one of them ate once a day. Of the students who skipped meals in a day, 35 (70%) skipped breakfast, 10 (20%) skipped lunch, and 5 (10%) skipped dinner. The students gained nutritional knowledge from various sources, i.e., 4 (8%) from their friends, 23 (46%) from the media, 9 (18%) from their parents, 12 (24%) from the school, while 2 (4%) from other sources apart from the ones listed.

Table 1. Socio-demographic data from 50 students at Novena University participated in this study.

	Frequency	%
Gender		
Male	27	54
Female	23	46
Educational status		
1st year	13	26
2nd year	11	22
3rd year	10	20
4th year	16	32
Place of residence		
University hostel	37	74
Privately rented apartment	12	24
Other	1	2

On the food groups that should be consumed less to have good health, 13 (26%) students mentioned carbohydrates, 6 (12%) mentioned proteins, 5 (10%) mentioned vegetables and fruits, 22 (44%) mentioned fats and oils, while 4 (8%) said they did not know. When asked how many servings of fruits should be consumed daily, 11 (22%) students said one, 13 (26%) said two, 10 (20%) said three, 3 (6%) said four, and 13 (26%) did not know. They provided a similar response when asked about the servings of fruits and vegetables that should be consumed daily for optimal health.

On the food groups that should be consumed most to be healthy, 5 (10%) students mentioned carbohydrates, 9 (18%) mentioned proteins, 1 (2%) mentioned fats and oils, 28 (56%) mentioned vegetables and fruits, while 7 (14%) said that they did not know regarding the answer to the question. The researchers also sought to know the advice the students would give to someone trying to lose weight. Nineteen (38%) students said increasing activity level and reduce energy intake, 6 (12%) said

Table 2. Eating practice and nutritional knowledge of 50 students at Novena University participated in this study.

Questions	Response	Frequency	%
1. How many meals do you usually eat in a day?	One	1	2.0
	Two	25	50.0
	Three	15	30.0
	Four	9	18.0
28. If you skip a meal, which meal is it usually?	Breakfast	35	70.0
	Lunch	10	20.0
	Dinner	5	10.0
28. Where do you mostly get nutritional information?	Friends	4	8.0
	Media	23	46.0
	Parents	9	18.0
	School	12	24.0
	Others	2	4.0
4. Which food group should you eat less according to your nutritional knowledge?	Carbohydrates	13	26.0
	Proteins	6	12.0
	Fats and oils	22	44.0
	Vegetables and fruits	5	10.0
	Do not know	4	8.0
5. Which food group should you eat the most?	Carbohydrates	5	10
	Proteins	9	18.0
	Fats and oils	1	2.0
	Vegetables and fruits	28	56.0
	Do not know	7	14.0
6. How many servings of fruits and vegetables should you have daily?	One serving per day	11	22.0
	Two servings per day	13	26.0
	Three servings per day	10	20.0
	Four servings per day	3	6.0
	Do not know	13	26.0
7. What advice would you give to someone trying to lose weight?	Increasing activity level and reducing energy intake	19	38.0
	Eating more food containing highly processed carbohydrates	6	12.0
	Replacing fat-free foods with whole grains	9	18.0
	Fasting all time	2	4.0
	Do not know	14	28.0

eating foods with highly processed carbohydrates, 9 (18%) said replacing fat-free foods with whole grains, while 14 (28%) had no advice to give.

As seen in Table 3, the minimum weight recorded for the students was 45 kg and the maximum was 95 kg. The mean was 67.1 kg. In terms of height, the tallest student was 201 cm and the shortest was 135 cm,while the mean height recorded was 167.9 cm. The BMI of the students ranged from 15 to 39, with amean and standard deviation of 23.93±5.46.

Table 3. Anthropometric data of 50 students at NovenaUniversity participated in this study.

Parameters	Min.	Max.	Mean	Standard deviation
Weight	45.00	95.00	67.10	14.57
Height	135.00	201.00	167.99	15.49
BMI	15.00	39.00	23.94	5.46

A cross-tabulation analysis was conducted to determine the potential relationship between nutrition knowledge and BMI using the designed questionnaire and the BMI of the students (Table 4). The Pearson's Chi-square critical value of 144.171 was bigger than the tabulated value, indicating a positive correlation between the students' nutritional knowledge and their BMI results. There was no significant difference between the students' nutritional knowledge and their BMI (P>0.005).

Table 4. Association between nutritional knowledge and BMI among students at Novena University.

	Value	df	Asymptotic significance (2-sided)
Pearson's Chi-square	144.171	140	0.387
Likelihood ratio	112.776	140	0.956
N of valid cases	50		

Table 5 presents the frequency and percentage of the students' BMI. There were 14% underweight students, 52% normal-weight students, and 22%

overweight students. Of the obese students, 8% had class I obesity and 4% had class II obesity.

Table 5. Classification of Novena University students' body weights according to the BMI formula.

BMI categories	Frequency	%
Underweight	7	14
Normal weight	26	52
Overweight	11	22
Class I obesity	4	8
Class II obesity	2	4

DISCUSSION

As noted by Ilori & Sanusi (2022), it is known that a high level of nutritional knowledge influences nutritional intake and eating practices. Similar to the BMI, anthropometric indices are one of the various parameters used in determining an individual's health status (Kearns et al. 2014). In addition to weight and height, the waist circumference may indicate a systemic inflammatory state in persons with obesity (Widjaja et al. 2019). Thus, the correlation between BMI and the nutritional knowledge of the studentswas examined.

The majority of the students resided in the university hostel and were therefore responsible for their daily meal preparation. It was a significant factor in their food selection and eating habits. Many students might also be frequent visitors to food vendors, where they purchased foods that would likely not satisfy them at a higher price than if they prepared it themselves. Thus, it was not surprising that half of the students ate only twice a day. The percentage of students in this study who consumed three meals a day was lower than the percentage recorded by (van den Berg et al. 2012). This study also revealed that breakfast was the most frequently skipped meal. It is linear with a study by Shimokawa (2013), who reported that the majority of Japanese university students ate three meals a day but frequently skipped breakfast. Another study by Sila et al. (2019) found that university students in Croatia frequently skipped breakfast.

The majority of the students in this study acquired nutritional knowledge from the media. This result is consistent with the findings of a study by Roemling & Qaim (2012) that the media is the primary source of nutritional knowledge for the population studied. In contrast, Chen et al. (2018) discovered that 46% of college students gained the majority of their nutritional knowledge from lectures. The sample size of the study, as well as the fact that media exposure and amount of time spent in front of television among the respondents can influence the amount and type of food consumed, may be the factors contributing to the discrepancy in the results (Teixeira et al. 2016, Chen et al. 2018).

The majority of the students identified carbohydrates, fats, and oils as the food groups that should be consumed less in order to maintain good health, whichare the correct answers. In contrast, when asked about the food groups that should be most frequently consumed, the majority of the students identified vegetables and fruits, while only a few did not know. This indicated that the majority of the students had a solid understanding of nutrition in relation to the food groups that should be consumed the most.

This study discovered that nutritional knowledge was positively correlated with BMI. This finding corresponds with the number of students who correctly identified the food groups to consume less or more of. The majority of the students were unaware ofthe daily serving recommendations for fruits and vegetables groups. Consequently, despite the fact that more than half of the students were of normal weight, some were overweight and obese. Those who knew what to eat according to the nutritional recommendation were more likely to maintain a healthy weight.

Strength and limitations

This study provides an insight of the correlation between nutritional knowledge and BMI. The findings of this study can help clinicians be aware that a healthy diet is vital, but so is nutritional knowledge. However, this study also has its limitations because the sample size is small compared to other studies andthe study population was not very diverse.

CONCLUSION

A high level of nutritional knowledge has an influence on dietary intake or eating practices. This affected the BMI and the onset of obesity among students at Novena University, Ogume, Nigeria. This study provides valuable justification for developing public policies to educate individuals about healthy eating habits and self-care in order to prevent the onset of obesity.

Acknowledgment

The authors sincerely appreciate the students, as well as the University Management of Novena University, Ogume, Nigeria, for providing a conduciveenvironment for this research.

Conflict of interest

None.

Ethical consideration

The Ethical and Research Committee of the Department of Anatomy, Novena University, Ogume, Nigeria, granted the ethical clearance for this study with the ethical code Novena/CHS/ANA/69/16 on 16/03/2020.

Funding disclosure

None.

Author contribution

UOA proofread the manuscript. OOG analyzed the data. ETM proofread the manuscript. DB collected the research data.

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Original Research Report

THE EFFECT OF *BINAHONG* (*Anredera cordifolia* (Ten.) Steenis) LEAF ETHANOLIC EXTRACT ON THE REDUCTION OF BLOOD URIC ACID LEVELS IN HYPERURICEMIC MALE WHITE WISTAR RATS (*Rattus norvegicus*)

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ABSTRACT

Binahong (*Anredera cordifolia* (Ten.) Steenis) is empirically used to treat burns, rheumatism, gout, typhoid, and stroke. *Binahong* leaves contain flavonoids that have an antioxidant effect. This study aimed to identify and test *binahong* leaf flavonoid compounds' antioxidant properties in reducing uric acid levels. This study began by producing *binahong* leaf extract and then identifying the flavonoid content with a phytochemical test. The results indicated that the *binahong* leaf extract contained antioxidant flavonoid compounds. The data were followed by testing flavonoid activity in lowering uric acid levels. This study used male white Wistar rats, of which the uric acid was induced by chicken liver juice. The animals used in the test were 25 rats divided into five groups (i.e., negative control group, positive control group, and treatment groups with dose I, dose II, and dose III). In each group, there were five male white rats. This study was a laboratory experiment using a pre-test post-test design with control groups (pre-test post-test control group design), where the grouping was done based on a randomized group design. By using statistical tests, the results obtained showed that there was a significant decrease of uric acid levels in the positive control group and the treatment group. In conclusion, *binahong* leaf ethanolic extract can reduce blood uric acid levels induced by chicken liver juice in male white Wistar rats.

Keywords: *Binahong*; blood uric acid; hyperuricemia; gout; rats; medicine

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Article history
●Submitted 2 Dec 2022 ● Revised 10 Jan 2023 ● Accepted 18 Feb 2023 ● Published 10 Mar 2023

How to cite: Anugerah P & Rahman S (2023). The effect of *binahong* (*Anredera cordifolia* (Ten.) Steenis) leaf ethanolic extract on the reduction of blood uric acid levels in hyperuricemic male white Wistar rats (*Rattus Norvegicus*). Folia Medica Indonesiana, 59 (1), 20-25. https://doi.org/10.20473/fmi.v59i1.36052

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. Antioxidant flavonoid compounds can be found in *binahong* leaf extract.
2. *Binahong* leaf extract is as effective as allupurinol in reducing uric acid levels.

INTRODUCTION

Gout arthritis is an inflammatory disease caused by the accumulation of uric acid crystals in synovial fluid and other tissues. Generally, the clinical manifestations of gout are pain, stiffness, and swelling in the joints. Gout usually occurs in the big toe, ankle, heel, elbow, wrist, and finger joints. Hyperuricemia is an early occurrence of gout, with increased blood uric acid levels reaching more than 7.0 mg/dl. The higher the uric acid level, the higher the risk of gout. Uric acid levels are influenced by age, sex, weight, and serum creatinine levels (Ragab et al. 2017, Centers for Disease Control and Prevention 2020).

The hyperuricemia incidence varies in each country. The asymptomatic hyperuricemia prevalence rate is 5% in the United States, 6.6% in England, and 8% in Scotland. In New Zealand, hyperuricemia is more common among Maori men (27.1%) compared to European men (9.4%). In a study conducted among Atayal people in Taiwan, it was found that 41.4% of 342 residents over 18 years old had hyperuricemia (George & Minter 2022).

The specific prevalence of hyperuricemia in Indonesia is unknown, but the data from several

studies in various regions showed a high incidence. The incidence of hyperuricemia in Sinjai, South Sulawesi, was 10% in men and 4% in women (Manampiring 2010). A survey conducted on 4,683 samples aged between 15-45 years in Bandungan, Central Java, found that the prevalence of hyperuricemia was 24.3% in men and 11.7% in women (Ladeska et al. 2018). The proportion of hyperuricemia in Tegal increased from 5.7% in 2007 to 8.7% in 2008. In the 2008 medical record data from Kardinah General Hospital, 40% of 1,068 patients who had their uric acid levels checked suffered from hyperuricemia (Prabawa 2019).

Hyperuricemia is an increase in blood uric acid above normal levels. Increased synthesis of purines into uric acid (overproduction) or decreased elimination of uric acid by the kidneys (underexcretion) can lead to hyperuricemia (Maiuolo et al. 2016). Unfavorable lifestyles, such as high purine intake and alcohol consumption, affect the incidence of gout. Genetic disorders of metabolism also play a role in causing excess uric acid production or decreasing uric acid excretion (Kanbay et al. 2016).

Treatment of gout is divided into acute attack management and chronic attack treatment. There are mainly three stages in the treatment of this disease: 1) overcoming acute attacks, 2) reducing uric acid levels to prevent the accumulation of uric acid crystals in the tissues and joints, and 3) using hypouricemic drugs to prevent hyperuricemia and gout from worsening. Patients must comprehend the fundamentals of therapy in order for gout treatment to be effective. Avoiding risk factors that can trigger attacks is an essential part in the management of this disease (Engel et al. 2017).

Allopurinol is the hypouricemic drug of choice for treating chronic gout caused by the overproduction of uric acid. Whereas, probenecid is the uricosuric drug of choice for treating gout caused by underexcretion of uric acid. Allopurinol functions as an inhibitor of xanthine oxidoreductase, an enzyme that prevents the formation of uric acid crystals by xanthine oxidase (Seth et al. 2014). However, allopurinol has various side effects, including hepatitis, gastrointestinal intolerance, and allergic reactions. Therefore, a new inhibitor of xanthine oxidoreductase as an alternative to allopurinol is needed (Qurie et al. 2022).

The main objective of this research was to find a new xanthine oxidoreductase inhibitor, with the same inhibitory activity as allopurinol and fewer side effects. The idea used was that utilizing natural ingredients is known to be safer for human consumption (Astini et al. 2017, Asari & Sugiyanta 2021). Indonesia's abundant biodiversity, if applied with the proper technology, can provide a competitive advantage in the global competition, particularly in the use of traditional medicinal plants (Hamdan et al. 2019, Astuti et al. 2020).

Binahong, also known as Madeira vine or mignonette vine, is one of Indonesia's abundant medicinal plants. Empirically almost all parts of *binahong* plant can be used for treatment, including lung disease, diabetes mellitus, hemorrhoids, dysentery, burns, and gout (Sakti et al. 2019). The plants contain many active compounds such as flavonoids (Dadiono & Andayani 2022). These compounds are abundant in *binahong* leaves, where leaf fertility is advantageous for obtaining high levels of active compounds (Rohani 2021). Antioxidant flavonoids can inhibit xanthine oxidase activity, thereby preventing the formation of uric acid. In addition to flavonoids, oleanolic acid has anti-inflammatory properties (Serrano et al. 2020). The active compounds in *binahong* are believed to be an alternative hypouricemic drug that reduce uric acid levels with fewer side effects than synthetic drugs like allopurinol (Engel et al. 2017).

MATERIALS AND METHODS

This study was a laboratory experiment employing a pre-test post-test design with control groups, where the grouping was determined by a randomized block design and the sample size was determined by a completely randomized design. This study employed male Wistar-strain white rats (*Rattus norvegicus*). The rats were randomly divided into five groups. All groups received the same diet for seven days to induce an increase in uric acid levels. Each group was fed chicken liver juice, pellets, and drinking water during the study (Sakti et al. 2019).

The minimum sample size was determined using the Federer formula as follows: $(n-1)(t-1)>15$. The number of the treatment groups is symbolized by “n”, while the number of replications or number of samples per group is symbolized by “t”. In this experiment, the minimum number of samples required per group was as follows: $(n-1)(5-1)>15$; $t=5$. It produced a result of $n>4.75$, with $t=5$. Based on the calculation, the number of samples per group must exceed 4.75. Therefore, five rats per group were utilized in this study. A total of 25 rats were required for the five groups studied. The rats were considered hyperuricemic when their blood uric acid levels exceeded 3.0 mg/dl (Engel et al. 2017).

The administration of *binahong* leaf extracts is classified into low, medium, and high doses. The dosage for the extract administration in humans was 50 mg/kg bw for the low dose, 100 mg/kg bw for the medium dose, and 200 mg/kg bw for the high dose.

These doses were converted for animal administration using the following method: converted dose for the test animal=absolute dose x conversion value. The conversion value for the rats in this study was 0.01836. The following are the results of the formula that served as a reference for conducting this experiment: 0.9 mg for the low dose (dose I), 1.8 mg for the medium dose (dose II), and 3.6 mg for the high dose (dose III). Distilled water was used to liquify the extracts, which were then administered orally using a gastric probe (Sakti et al. 2019).

The standard therapeutic dose of allopurinol for hyperuricemia in the general population is 300 mg/50 kg bw. Therefore, the allopurinol dose for humans as a reference in this research was 300 mg per day. The conversion value for dose conversion between human and rats was 0.01836. The converted allopurinol dose for the rats was 5.4 mg/200 g bw (Sakti et al. 2019).

Twenty-five rats, previously divided into five groups with each group consisted of five rats, were induced by chicken liver juice. Chicken liver contains purines of 150-1,000 mg/100 g. The chicken liver juice dose was 3 ml/200 g bw, adjusted to the maximum fluid intake capacity of the rats, which was 5 ml/200 g bw. Group I (positive control group) was fed chicken liver juice, distilled water, and allopurinol with a dose of 5.4 mg/kg bw. Group II (negative control group) was fed chicken liver juice and distilled water. Group III (treatment group I) was fed chicken liver juice, distilled water, and *binahong* leaf extract with a dose of 0.9 mg. Group IV (treatment group II) was fed chicken liver juice, distilled water, and *binahong* leaf extract with a dose of 1.8 mg. Group V (treatment group III) was fed chicken liver juice, distilled water, and *binahong* leaf extract with a dose of 3.6 mg (Ladeska et al. 2018).

The normal distribution of the data obtained was assessed using one-sample Kolmogorov-Smirnov test and the Shapiro-Wilk test, while the homogeneity of the data was assessed using using the Levene test. If these two conditions were met, a one-way ANOVA test would be performed to determine whether or not there was a significant difference among the treatment groups (Ladeska et al. 2018). If there was a significant difference, then a difference test using the Duncan method would be conducted. However, if one or both of these tests were not met, the analysis would be carried out using the Kruskal-Wallis test. Povidone-iodine was used to close and dry the wound from a previous blood draw on the tail. After blood samples were taken, the experimental animals were returned to their cages (Sakti et al. 2019).

RESULTS

Data on the blood uric acid levels before and after the experiment from all groups were tested using the Smirnov test for data normality and the Levene test for homogeneity. The data are normally distributed and equal in variances if p=0.05 in each test. One-way ANOVA test can be conducted on normally distributed and homogeneous data. If p=0.05 in the ANOVA test, then a difference test must be carried out using the Duncan method.

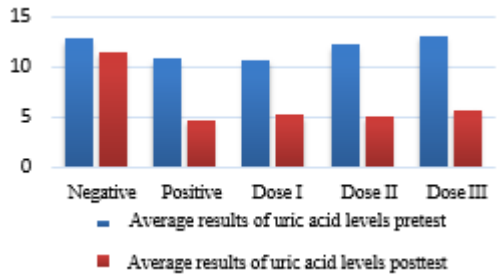

Figure 1. Average results of uric acid levels in all groups.

The analysis of the data resulted in a significance value of 0.05, so the data were considered homogenous. The decrease in uric acid levels in the rats from all groups was normally distributed. Therefore, the data can be continued to be analyzed in the ANOVA test.

Table 1. Results of the ANOVA test on data of the rats' uric acid levels.

F Count	F table 0.05	F table 0.01	p-value
3.846	2.87	4.43	0.018

The results of the ANOVA test ($p<0.05$) indicated that uric acid levels in the control and treatment groups decreased significantly after treatment, with the calculated F value exceeding the F table value. The data showed a significant decrease, so a difference test using the Duncan method was conducted. The purpose of the difference test was to compare the differences among the animal groups.

The conclusion from the analysis results was that the decrease in uric acid levels among the negative control group, the positive control group, and the treatment groups differed significantly. However, the decrease in uric acid levels between the positive control group and the treatment groups did not differ significantly ($p>0.05$). The treatment group I with the highest dose of *binahong* leaf extract demonstrated

the greatest reduction in uric acid levels. The positive control group, treatment group II, and treatment group III followed suit.

Table 2. Results of the least significant difference test using the Duncan method.

Group	N	1	2
Negative control	5	1.300	
Treatment I	5		5.140
Treatment II	5		5.680
Positive control	5		6.000
Treatment III	5		7.400
P-value		1.000	0.223

DISCUSSION

The results of this study showed an increase in uric acid levels among the rats in the control and treatment groups after being fed chicken liver juice for seven days. It was because chicken liver juice contains high levels of purines, which triggered xanthine oxidase to catalyze the formation of uric acid. *Binahong* leaf extract administration could reduce uric acid levels of experimental animals, i.e., male white rats (*Rattus norvegicus*) induced by chicken liver juice. The results of the ANOVA test ($p<0.05$) proved that there was a significant average difference between the control and treatment groups. The presence of essential compounds in *binahong*, such as flavonoids, was assumed to play a role in lowering uric acid levels (Darmawan & Hidayati 2020).

This research is also supported by several other studies. Ablat & Mohamad (2018) reported in their study that flavonoids reduce uric acid levels by inhibiting the enzyme xanthine oxidase, which is responsible for the formation of uric acid. The types of flavonoids that can inhibit the activity of xanthine oxidase are quercetin, myricetin, kaempferol, luteolin, apigenin, and chrysin. Nadinah, as cited by Martha & Zummah (2018), stated that luteolin and apigenin can work as xanthine oxidase inhibitors with similar performance as allopurinol.

Allopurinol was used as a comparison drug because it is a modern medication commonly used to reduce uric acid levels. It is believed that this nucleic acid derivative can inhibit the production of uric acid. This inhibitory mechanism of allopurinol is used to maintain stable uric acid synthesis. With *binahong* leaf extract, the flavonoids and xanthine oxidase interact to make the bonds loose, then stabilize the xanthine oxidase (Martha & Zummah 2018).

The normality test (one-sample Kolmogorov-Smirnov test) showed that the data on the blood uric acid levels of all groups were normally distributed ($p\geq0.05$). The homogeneity test (Levene test) showed homogeneous variance ($p\geq0.05$). The analysis can be continued with an ANOVA test. If $p\leq0.05$, a difference test can be carried out using the least significant difference and Duncan method.

In the difference test for all groups, the positive control group and treatment groups showed a significant difference ($p\leq0.05$) from the negative control group. All treatment groups showed no significant difference ($p\geq0.05$) from the positive control group. Even though the blood uric acid levels in all treatment groups and the positive control group were not normal, there was a decrease in uric acid levels compared to the negative control, and the the results in all treatment groups were comparable to that of the positive control group. Based on the findings of this study, it can be concluded that increasing the dose concentration of the *binahong* leaf ethanolic extract correlates significantly with the extract's efficacy in lowering total cholesterol levels. The treatment groups I, II, and III showed a significant difference in the reduction of total cholesterol levels. Comparing the decrease in uric acid levels between the treatment and control groups illustrated this point.

Strength and limitations

The study provides an insight on the identification and testing the of antioxidant activity of *binahong* leaf flavonoid compounds in reducing uric acid levels and discovering a novel xanthine oxidoreductase inhibitor that has the same inhibitory effect as allopurinol but with fewer adverse effects. The study about *binahong* has not been widely explored in a lot of other studies.

CONCLUSION

The administration of *binahong* (*Anredera cordifolia* (Ten.) Steenis.) leaf extract for three days can decrease uric acid levels. *Binahong* extract's effectiveness in reducing uric acid levels is comparable to that of allopurinol, and it is even more effective at the third dose. The higher the *binahong* leaf extract dose administered, the bigger the reduction in uric acid levels.

Acknowledgment

We would like to thank the Orthopedic and Traumatology Laboratory, as well as the Orthopedic and Traumatology Specialist Medical Education Program, Faculty of Medicine, Syiah Kuala

University, Banda Aceh, Indonesia, for their assistance in carrying out this study.

Conflict of interest

None.

Ethical consideration

An ethical approval was obtained from the Research Ethics Committee, Syiah Kuala University, Banda Aceh, Indonesia (No. 225/KE/FK/2014 on 23/09/2014).

Funding disclosure

None.

Author contribution

PA was in charge of the study's design, data collection, and data analysis. SR made necessary intellectual revisions to the work and gave the final draft his approval for publication.

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Original Research Report

PERCUTANEOUS NEPHROLITHOTOMY (PCNL) IN OLDER AND YOUNGER PATIENTS AT A TERTIARY HOSPITAL IN SURABAYA, INDONESIA

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ABSTRACT

Percutaneous nephrolithotomy (PCNL) for renal and proximal ureteral stone treatment among the elder population is considered challenging due to the complication risk associated with comorbidity and lower functional reserve. Patients older and younger than 60 years old were compared for efficacy and safety in following PCNL procedures. Consecutive patients who underwent PCNL from 2019-2021 in a single center were divided into patients aged at least 60 years (group I) and patients aged under 60 years (group II). Single stage fluoroscopic-guided PCNL were used for the entire study population. Patients' habitus, stone-related, and operative characteristics were compared. The two groups' PCNL success and complication rates were evaluated. A total of 245 patients, comprising 65 in group I and 180 in group II, were included for analysis. Diabetes mellitus prevalence was higher in group I (30.8% vs 18.9%). However, the study population did not show a significant difference in regard to comorbidity. Operative time, success rate (80% vs 74.4%), and complication rate (16.9% vs 15.6%) did not statistically differ (p>0.05). Transfusion rate was higher among patients aged at least 60 years (p=0.018). Based on the multivariate analysis, stones located in the renal pelvis was the factor which contributed to the success rate. In conclusion, percutaneous nephrolithotomy is a safe and effective procedure for treating renal and proximal ureteral stones in the older population. Blood transfusions are more frequently given, in part, due to bleeding risk among older patients.

Keywords: Percutaneous nephrolithotomy (PCNL); renal stone; nephrolithiasis; older adults; life expectancy

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Article history
●Submitted 20 Dec 2022 ● Revised 20 Jan 2023 ● Accepted 23 Feb 2023 ● Published 10 Mar 2023

How to cite: Ali WM, Azmi YA, Tarmono, et al (2023). Percutaneous nephrolithotomy (PCNL) in older and younger patients at a tertiary hospital in Surabaya, Indonesia. Folia Medica Indonesiana, 59 (1), 26-31. https://doi.org/10.20473/fmi.v59i1.40968

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. Percutaneous nephrolithotomy (PCNL) in older patients is as effective and safe as in younger patients.
2. It is a viable option for managing renal stones in older patients with indications, although blood transfusions are frequently required.

INTRODUCTION

Nephrolithiasis, commonly referred to as kidney stones, is a urological disease that causes high morbidity. One problem that arises from nephrolithiasis is the high comprehensive and long-term treatment cost. In addition to the expensive cost, advanced nephrolithiasis might result in death due to kidney failure (Tang & Lieske 2014). In Indonesia, 6 out of 1,000 people have kidney stones, indicating the prominence of the disease given the country's population of more than 250 million people. This places kidney stones as the third most frequent urological disease. Considering the high incidence rate of kidney stones among people aged 30-50 years, it is clear that this age group is particularly prone to the disease, and the incidence in men is three times that of women (Kurniawan et al. 2020). Furthermore, considering that calcium oxalate is responsible for the majority of stone occurrences, it demonstrates that environmental factors have a significant impact. In Indonesia, there

is a region known as the "stone belt", which has a high incidence of kidney stones. This phenomenon has occurred due to the high calcium levels in the drinking water (Lestari et al. 2019, Kurniawan et al. 2020).

Percutaneous nephrolithotomy (PCNL) is one of the therapeutic modalities that can be considered to destroy stones in the kidneys and the renal pelvis (Wicaksono et al. 2022). The advantage of PCNL over kidney stone therapy is that it can destroy stones larger than 20 mm, which lithotripsy cannot (Ziemba & Matlaga 2017).

According to demographic data, the age at which kidney stones occur varies substantially among older and younger patients (Rule et al. 2014). Because PCNL is an invasive procedure, the patient's age is important to consider, especially if the patient is very old and demonstrates changes in body structure and posture. Until recently, no known research had investigated the relationship between age and PCNL, so it was still unclear whether the PCNL method could be performed safely for all ages. Therefore, this research investigated the relationship between age and the PCNL procedure.

MATERIALS AND METHODS

This research was a descriptive study with a retrospective cohort design. Data were obtained in single sampling period at the Central Medical Record Unit, Department of Urology, and the Operating Room of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The sample in this study was the medical records of all patients with a diagnosis of kidney stones who received PCNL treatment at the Urology Department of Dr. Soetomo General Academic Hospital from January 2019 to December 2021. The sampling technique used was total sampling.

The data collection was carried out by tracing the patients' polyclinical status and medical records at Dr. Soetomo General Academic Hospital. The data were then gathered based on the inspection and actions performed. Data collected included the number of patients, age, comorbidities, complications, and outcomes of PCNL measures. The information gathered comprised the number of patients, their ages, comorbidities, complications, and the outcomes of the PCNL procedure. IBM SPSS Statistics for Windows Version 21.0 (IBM Corp., Armonk, N.Y., USA) was used to examine the statistical data. Tables, figures, and narratives in this article are used to present the research findings in a descriptive manner.

RESULTS

From January 2019 to December 2021, 245 patients who underwent PCNL procedure at Dr. Soetomo General Academic Hospital provided complete data and met the inclusion criteria of this study. The PCNL was performed in accordance with established procedures at Dr. Soetomo General Academic Hospital, and the evaluation occurred two weeks to three months after the procedure. The patients were then divided into two age groups: the older (≥60 years old) and the younger (<60 years old). A total of 245 patients met the inclusion criteria for this study. Table 1 shows the preoperative characteristics of the research sample.

Table 1. Preoperative characteristics of the patients in the older andyounger age groups.

Characteristics	≥60 y.o. (n=65)	≤60 y.o. (n=180)	p-value
Age			
Average (SD)	65.02 (4.01)	45.51 (12.10)	
Range (min-max)	60-80		
Comorbidity			
Diabetes mellitus	20 (30.8%)	34 (18.9%)	0.071
Hypertension	36 (55.4%)	114 (63.3%)	0.328
BMI			
Average (SD)	20.84 (2.96)	20.99 (2.95)	0.842
Stenting & nephrostomy			0.393
Stenting	6 (9.2%)	26 (14.4%)	
Nephrostomy	5 (7.7%)	13 (7.2%)	
Side			0.886
Right	38 (58.5%)	110 (61.1%)	
Left	27 (41.5%)	70 (38.9%)	
Size			0.822
Average (cm) (SD)	2.45 (1.6)	2.39 (1.5)	
Location			0.691
Staghorn	14 (21.5%)	37 (20.6%)	
Renal pelvis	17 (26.2%)	60 (27.8%)	
Caliseal	23 (35.4%)	50 (33.3%)	
Renal pelvis & caliseal	8 (12.3%)	28 (15.6%)	
Ureteropelvic junction	1 (1.5%)	4 (2.2%)	

According to Table 1, there were 65 patients (26.5%) aged 60 and older, and 180 patients (44.08%) under the age of 60. The patients' average

age in the older group (≥60 years old) was 65.02±4.01, while it was 45.51±12.10 in the younger group (<60 years old). Diabetes mellitus was the comorbidity that exhibited a significant difference between the older and younger age groups, with 30.8% of the older patients having diabetes mellitus compared to 18.9% in the younger age group (p=0.071).

There was a substantial difference in postoperative characteristics, with 16.9% of the older patients requiring blood transfusions compared to 6.1% of the younger patients (p=0.018) (Table 2). According to the data presented above, the stone-free rates were 73.8% (48 out of 65 cases) in the older age group and 68.3% (123 out of 180 cases) in the younger age group. There was no significant difference in the stone-free rates between the older and younger groups, according to the results of statistical analysis using the Chi-square test (p=0.501).

Table 2. Postoperative characteristics of the patients in the older and younger age groups.

Characteristics	≥60 y.o. (n=65)	≤60 y.o. (n=180)	p-value
Success rate	52 (80%)	134 (74.4%)	0.466
Stone-free rate	48 (73.8%)	123 (68.3%)	0.501
Complication rate	11 (16.9%)	28 (15.6%)	0.952
Bleeding	11 (16.9%)	20 (11.1%)	
Blood transfusion	11 (16.9%)	11 (6.1%)	0.322
Decreased Hb			0.018
Average (SD)	1.34 (0.75)	1.17 (0.62)	0.261
Stone size			
Average (SD)	2.45 (1.57)	2.39 (1.50)	0.822
Puncture number			0.909
3	32 (49.2%)	93 (51.7%)	
4	21 (32.3%)	53 (29.4%)	
5	12 (18.5%)	34 (18.9%)	
Operation duration			
Average (SD)	114.51 (43.78)	116.31 (34.16)	0.472

In the older age group, 32 patients (49.2%) received three punctures, 21 (32.3%) received four punctures, and 12 (18.5%) received five punctures. Among the younger patients, 93 (51.7%) received three punctures, 53 (29.5%) received four punctures, and 34 (18.9%) received five punctures. The statistical analysis using the Chi-Square test revealed that the number of punctures was not significantly different between the older and younger age groups (p=0.909) (Table 2).

Table 2 shows that complications occurred in 11 (16.9%) of the 65 cases among the older patients. Complications were reported in 39 (15.9%) of 180 cases among the younger patients. The statistical analysis using the Chi-Square test showed no significant difference in the post-PCNL complications between the older and younger groups (p=0.796). The average duration of PCNL procedure in the older age group was 114.5±44.1 minutes, while it was 116.3±34.3 minutes in the younger age group. Statistical analysis employing the Mann-Whitney test revealed no statistically significant difference between the duration of the PCNL procedure in the older and younger groups (p=0.472).

Table 2 shows that the average stone sizes were 2.45±1.57 cm in the older age group and 2.39±1.50 cm in the younger age group. The results of statistical analysis using the Mann-Whitney test revealed no significant difference in the stone sizes between the older and younger groups (p=0.822).

DISCUSSION

Kidney stone (nephrolithiasis) is the most common type of urinary tract stone. Nephrolithiasis is caused by the formation of crystals in the kidneys (Alelign & Petros 2018). The prevalence of this condition varies with geography. Epidemiology study suggests that this disease is linked to a nation's socioeconomic welfare and development. It is more common in developed countries due to the influence of the population's nutritional status and daily activities. It also depends on the climate and temperature of an area (Purnomo 2016). Seasonality is associated with temporary changes in urine composition. When the air temperature rises, urine production decreases. Reduced urine production causes the ions in the urine to become concentrated, facilitating stone formation (Eisner et al. 2012).

Some Indonesian areas, known as the "stone belt", have a higher incidence rate of kidney stones than other areas. Those who reside in hot areas with high UV exposure tend to experience dehydration, increased vitamin D production that triggers higher excretion of calcium and oxalate, and profuse perspiration that can reduce urine production (Purnomo 2016). The prevalence of kidney stones in Indonesia is 0.6%, indicating that 6 out of every 1,000 people suffer from the disease (Minister of Health of the Republic of Indonesia 2013). The prevalence of nephrolithiasis is also increasing as a result of global warming, which elevates global

temperatures by 1-3 degrees Celsius (Goldfarb & Hirsch 2015). One of the modalities for nephrolithiasis is percutaneous nephrolithotomy (PCNL), which is the standard procedure for the endourological management of large nephrolithiasis. Many other aspects of surgical techniques and endoscopic equipments have improved since the introduction of this approach in 1976 (Carrion et al. 2018).

Data for this study were collected from 245 patients who met the inclusion criteria and underwent the PCNL procedure at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, from January to December 2019. The PCNL was performed in accordance with standard operating procedures at Dr. Soetomo General Academic Hospital, and the follow-up was conducted two weeks to three months later. The patients were categorized into two age groups: the older (≥60 years) and the younger (<60 years).

In this study, 65 of the 245 patients with kidney stones were over 60 years old, whereas 180 were under 60. Diabetes mellitus was the comorbidity that exhibited a significant difference between the older and younger age groups, with 30.8% of the older patients having diabetes mellitus compared to 18.9% of the younger patients (p=0.071). Similar findings were discovered in two prior studies by Abedali et al. (2019) and Buldu et al. (2015), in which diabetes mellitus was shown to be considerably more prevalent in the older age group investigated. In a study conducted by Wei et al. (2015), diabetes was found to be significantly associated with an increased frequency of postoperative and infectious complications following PCNL. Clinicians should note that approximately 50% of older patients present their first kidney stone episode at admission to emergency rooms, and the risk increases with the presence of diabetes mellitus (Arampatzis et al. 2012). A study even found that type 2 diabetes increased the risk of chronic kidney disease, especially in patients with high blood pressure and low high-density lipoprotein (Sutadji et al. 2023).

Blood transfusion were required in 16.9% of cases in the older age group compared to 6.1% in the younger age group (p=0.018). Abedali et al. (2019) found that preoperative hemoglobin (Hb) was considerably lower in the older age group (above 80 years) than in the other two groups (20-59 years and 60-79 years) (p<0.0001). Another study discovered that, when compared to other groups, people over the age of 80 had a 2 to 4 times higher risk of requiring blood transfusions post-surgery (Abedali et al. 2019). Although the majority of bleeding in PCNL procedures could be managed conservatively, severe bleeding in about 0.8% of cases required renal arteriography and selective embolization (Usawachintachit et al. 2016). The renal calyx was the most common location for PCNL based on the location of kidney stones. There was no statistically significant difference in the location of the stones between the two groups. Caliceal stones are typically accumulated in the major or minor calyces. Caliceal stones may be asymptomatic until they migrate to the ureters and induce ureteral colic pain (Gross et al. 2014).

The stone-free rates after the PCNL procedure did not differ significantly between the older and younger age groups, with 73.8% of the older patients and 68.3% of the younger patients being stone-free (p=0.501). This is similar to a study by Morganstern et al. (2015), in which stone-free rates were attained without a statistically significant difference (p=0.23) between the groups aged above 80 years (78%) and under 65 years (82%). For long-term management of kidney stones, clinicians must be aware that patients with kidney stones may have genetic, environmental, and nutritional risk factors that contribute to kidney stone recurrence. As a result, kidney stones are more likely to recur if the patients do not implement lifestyle adjustments (Han et al. 2015). On the other hand, poor renal function and more than seven days of bed rest were risk factors for malnutrition (particularly protein deficiency) in older patients (Zurriyani et al. 2020). Following the PCNL procedure, residual stone may also be present and should be removed with an additional procedure, such as extracorporeal shock wave lithotripsy (Sawal & Soebadi 2020).

The frequencies of complications following the PCNL were 16.9% in the older age group and 15.6% in the younger age group. There was no significant difference in the occurrence of post-PCNL complications between these two groups. Nakamon et al. (2013) discovered no significant difference in post-PCNL complications between the older age group (over 65 years) and the younger age group (less than 65 years), with the exception of sepsis, which occurred at 6.56% in the older age group and only 1.3% in the younger age group (p=0.007). Another study found that patients over the age of 80 who underwent PCNL had no long-term complications or fatalities (Meng et al. 2019).

In this study, the average durations of PCNL procedure were 114.5±44.1 minutes in the older patients and 116.3±34.2 minutes in the younger patients. The Kolmogorov-Smirnov test results revealed that the data were normally distributed. A study by Nakamon et al. (2013) also reported no difference in the duration of surgery between the older age group (over 65 years) and the younger age group (under 65 years). There was no significant difference in stone complexity between the older and younger age groups in terms of the affected

kidney (p=0.886), size (p=0.822), or location (p=0.691).

Strength and limitations

Because the two groups' stones were similar in complexity, this study could provide a statistically valid comparison of postoperative outcomes. The limitations of this study were attributable to the nature of a retrospective study and the minimal number of samples included. Further prospective research with a larger sample size may strengthen the findings of this study.

CONCLUSION

Percutaneous nephrolithotomy (PCNL) in older patients is as effective and safe as in younger patients, albeit with a higher probability of comorbidities in older age. PCNL is a viable management option for older adults with indications. However, older patients may require more blood transfusions during a PCNL procedure than younger patients. Further studies, particularly with a prospective approach and a larger sample size, are important to support the findings of this study and reduce the likelihood of bias.

Acknowledgment

The authors thank the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, for supporting this study.

Conflict of interest

None.

Ethical consideration

This study was approved by the Health Research Ethics Committee at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, with reference number 1101/LOE/301.4.2/X/2022.

Funding disclosure

This research was funded by the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. The benefactor had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contribution

MWA, YAA, T, and DMS conceptualized the study, conducted the investigation and validation, as well as wrote, reviewed, and edited the manuscript. MWA and YAA conducted the data curation and formal analysis, as well as drafted the original manuscript. MWA, T, and DMS contributed to the methodology, while MWA also provided the resources. T and DMS supervised the study.

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Original Research Report

THE USE OF STRONGkids, TOTAL LYMPHOCYTE COUNT, AND SERUM ALBUMIN TO IDENTIFY THE RISK OF HOSPITAL MALNUTRITION IN CHILDREN

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ABSTRACT

Hospital malnutrition occurs in hospitalized patients who do not consume enough food while their nutritional requirements increase. It occurs particularly in children who have undergone gastrointestinal surgery. Despite the lack of a universal instrument for detecting hospital malnutrition, various parameters can be considered to assist in its identification. STRONGkids has demonstrated its efficiency in detecting malnutrition risk in children. Total lymphocyte count (TLC) and serum albumin are biochemical markers that are related to infection and protein leakage, which can worsen hospital malnutrition. The research objective was to analyze the correlation between STRONGkids and biochemical markers (TLC, serum albumin) to identify hospital malnutrition in children who underwent gastrointestinal surgery. This was a retrospective cross-sectional observational study utilizing medical records. The statistical analysis was conducted using SPSS 21. This study included 37 subjects, with a 24.32% hospital malnutrition incidence rate. The subjects were divided into two groups: hospital malnutrition (n=9) and non-hospital malnutrition (n=28). The STRONGkids of both groups at admission demonstrated a significant difference, while the albumin and TLC did not. The significantly different STRONGkids scores of both groups at admission correlated negatively with the length of hospital stay (LOS), body weight reduction, TLC, and albumin. Those parameters also did not correlate with hospital malnutrition. However, hospital malnutrition increased the risk of low albumin and TLC at discharge by 2.951 and 5.549 times, respectively. In conlusion, TLC and serum albumin cannot be used as independent markers for hospital malnutrition, but STRONGkids can be used in conjunction with TLC and serum albumin to identify hospital malnutrition risk.

Keywords: Total lymphocyte count; serum albumin; STRONGkids; hospital malnutrition; children

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Article history
●Submitted 20 Nov 2022 ● Revised 15 Dec 2023 ● Accepted 25 Feb 2023 ● Published 10 Mar 2023

How to cite: Rusti HA, Widjaja NA, Irawan R, et al (2023). The use of STRONGkids, total lymphocyte count, and serum albumin to identify the risk of hospital malnutrition in children. Folia Medica Indonesiana, 59 (1), 32-39. https://doi.org/10.20473/fmi.v59i1.40015

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. Medium- and high-risk STRONGkids scores are related with low total lymphocyte count and serum albumin, which are related to hospital malnutrition, albeit indirectly.
2. The use of STRONGkids with total lymphocyte count and serum albumin can detect the risk of hospital malnutrition in children.

INTRODUCTION

Hospital malnutrition refers to malnutrition that occurs during hospital treatment (Juliaty 2016). Hospital malnutrition is a common and undertreated condition in oncology patients, leading to longer hospital stays and higher healthcare costs (Planas et al. 2016). Hospital malnutrition prevention and treatment represents a tremendous opportunity to improve overall patient care quality, clinical outcomes, and cost-effectiveness (Tappenden et al. 2013). The hospital malnutrition prevalence in children ranges from 6.1% to 51.6%, depending on the population studied and the diagnostic criteria

used. The data are supported by the proportion of hospitalized children with malnutrition at several Indonesian hospitals, including Dr. Sardjito Central General Hospital, Yogyakarta (27%), Sanglah Central General Hospital, Denpasar (31.5%), Saiful Anwar General Hospital, Malang (24.3%), and Mohammad Hoesin Central General Hospital, Palembang, Indonesia (37%) (Maryani et al. 2017). Patients undergoing abdominal surgery are typically susceptible to malnutrition. This is due to dietary restriction, surgical stress, long periods of starvation before and after surgery, and an increase in metabolic rate after surgery (Permsombut et al. 2013). A study utilizing anthropometric measurements from the World Health Organization (WHO) for the pre-operative period found that the prevalence of malnutrition was 46.2% among children admitted for elective general surgery in Nigeria (Adigun & Ogundoyin 2020).

Malnutrition is significant in children who have had gastrointestinal surgery because it increases complications from the primary disease, resulting in local infection, systemic infection, additional surgery intervention, and an extended length of hospital stay (LOS) (Koofy et al. 2021). Malnutrition in children is even significantly related to the incidence of pneumonia and stunting (Sekiyama et al. 2015, Wicaksono 2016). There are several interventions to prevent the condition, but it is unclear how well they are adopted by both malnourished and well-nourished children and their mothers, and to what extent socioeconomic factors have impacts (Tette et al. 2015). It is prevalent in pediatric patients with diarrhea and acyanotic congenital heart defect (Fedora et al. 2019, Jordan et al. 2020). Therefore, identifying malnourished patients, particularly in children, through hospital malnutrition risk assessment is important.

In daily practice, many nutritional screening tools (NST) are used to identify the risk of hospital malnutrition in children, but none is universally accepted (Lee 2018). Screening Tool for Risk on Nutritional Status and Growth (STRONGkids) is one of the most frequently used NST (Hulst et al. 2010). Rocha & Fortes (2015) found that in addition to NST, total lymphocyte count (TLC) and serum albumin should be considered as supporting biochemical parameters for identifying malnutrition. The European Society for Parenteral and Enteral Nutrition (ESPEN) and American Society for Parenteral and Enteral Nutrition (ASPEN) have recommended guidelines for nutritional risk screening to identify hospitalized patients at risk of malnutrition (Kondrup 2003, Thibault et al. 2021). With the provided information, fewer patients are expected to have to deal with the complications of nutritional imbalance despite also suffering from their underlying disease.

MATERIALS AND METHODS

This retrospective observational cross-sectional study was conducted from October 2021 to January 2022 at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. All subjects in this study were children aged 3 to 24 months who underwent gastrointestinal surgery at the Chlid Health Department of Dr. Soetomo General Academic Hospital between January 2016 and January 2021. All data were extracted from the medical records of the subjects. Subjects with a tumor, syndrome anomaly, dysmorphic facial features, down syndrome, or incomplete medical record were excluded from the study.

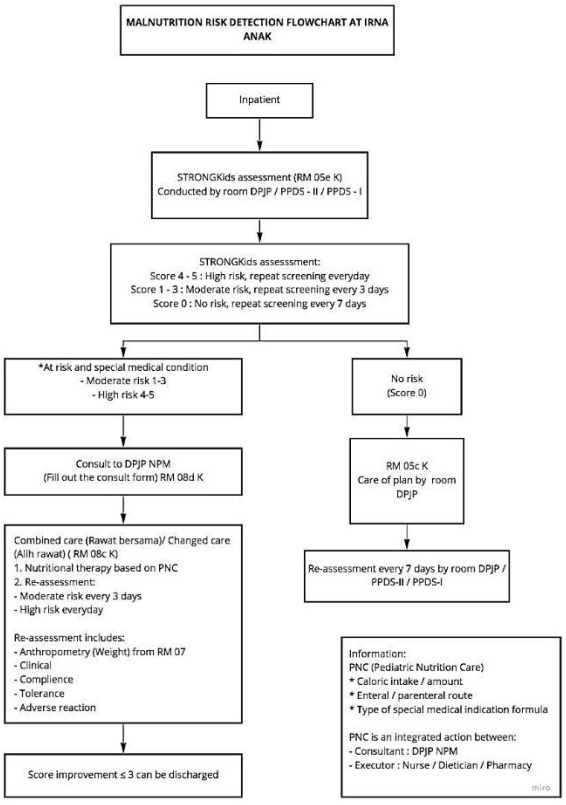
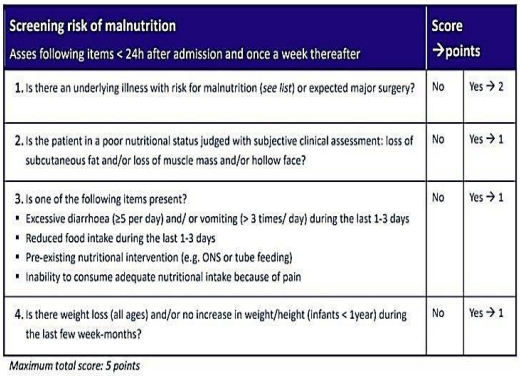


Figure 1. Flowchart of the malnutrition risk detection at Dr. Soetomo General Academic Hospital (Wahyuhadi 2018).

The data obtained from the study consisted of age, gender, serum albumin, total lymphocyte count, and STRONGkids score. As suggested by Maryani et al. (2017), this study defined hospital malnutrition as malnutrition that occurs during hospitalization, as indicated by a decrease in weight of >2% within 7 days, 5% within 8 to 30 days, or 10% within >30 days. The biochemical marker data from laboratory assessment were obtained from two distinct time periods, i.e., one from at least three days before the surgery and the other from at least three days after the surgery. All assessment results were collected by

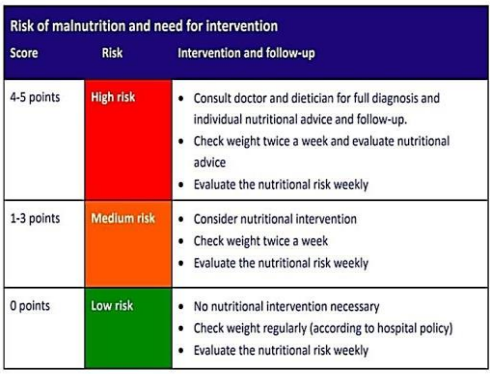
laboratory personnel. Serum albumin concentrations and total lymphocyte counts were the variables under investigation in this study. The cutoff point for normal serum albumin concentration in this study was consistent with previous research, whereas ≤3.00 g/dL was considered hypoalbuminemia.



Screening risk of malnutrition Asses following items < 24h after admission and once a week thereafter	Score →points	
1. Is there an underlying illness with risk for malnutrition (*see list*) or expected major surgery?	No	Yes → 2
2. Is the patient in a poor nutritional status judged with subjective clinical assessment: loss of subcutaneous fat and/or loss of muscle mass and/or hollow face?	No	Yes → 1
3. Is one of the following items present? ▪ Excessive diarrhoea (≥5 per day) and/ or vomiting (> 3 times/ day) during the last 1-3 days ▪ Reduced food intake during the last 1-3 days ▪ Pre-existing nutritional intervention (e.g. ONS or tube feeding) ▪ Inability to consume adequate nutritional intake because of pain	No	Yes → 1
4. Is there weight loss (all ages) and/or no increase in weight/height (infants < 1year) during the last few week-months?	No	Yes → 1

Maximum total score: 5 points

Figure 2. Factors and scores in the assessment of malnutritionusing STRONGkids (Rad 2019).



Risk of malnutrition and need for intervention		
Score	Risk	Intervention and follow-up
4-5 points	High risk	• Consult doctor and dietician for full diagnosis and individual nutritional advice and follow-up. • Check weight twice a week and evaluate nutritional advice • Evaluate the nutritional risk weekly
1-3 points	Medium risk	• Consider nutritional intervention • Check weight twice a week • Evaluate the nutritional risk weekly
0 points	Low risk	• No nutritional intervention necessary • Check weight regularly (according to hospital policy) • Evaluate the nutritional risk weekly

Figure 3. Interpretation of malnutrition risk according to the STRONGkids scores (Rad 2019).

The total lymphocyte count was calculated by combining the lymphocyte percentage and the leucogram value. The cutoff point for normal TLC was <3000 cells/m^3, according to a study by Tosato et al. (2015). A study on malnutrition in pediatric HIV patients also affirmed to use 3000 cells/m^3 as the cutoffpoint for TLC (Widjaja et al. 2016). The data were collected for analysis using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp. Armonk, N.Y., USA). The results were then assessed using the Spearman correlation test along with the Kolmogorov-Smirnov normality test. The Pearson correlation test confirmed the degree of association between the variables for the diagnosis without categories. In order to compare the various parameters, analyses were carried out using binary logistic regression, Chi-square, Fisher's exact, independent t-test, Mann-Whitney test, Spearman's rho, and Pearson'scorrelation, with $p<0.05$ indicated a significance.

RESULTS

A total of 37 samples retrieved from the medical records were eligible to fulfil all of the inclusion criteria. Nine subjects (24.3%) were found to have hospital malnutrition. Table 1 shows the characteristics of the hospitalized patients with malnutrition.

Table 1. General characteristics of the hospitalized subjects.

	HMN (n=9)	Non-HMN (n=28)	*p*
	Mean±SD	Mean±SD	
Age, months	8.78 ±3.31	11.00 ±8.17	0.986[1]
Sex, n (%)			
-Male	7 (77.78)	15 (53.57)	0.262[2]
-Female	2 (22.22)	13 (48.43)	
Diagnosis, n (%)			
-Ileal obstruction	3 (33.33)	8 (28.57)	
-Duodenal obstruction	2 (22.22)	6 (21.42)	
-Hirschsprung's disease	1 (11.11)	7 (25)	0.898[4]
-Intussusception	2 (22.22)	5 (17.85)	
-Hypertrophic pyloric stenosis	1 (11.11)	1 (3.57)	
-Volvulus	0 (0)	1 (3.57)	
LOS, day	24.33 ±27.23	28.07 ±22.92	0.687[3]
Weight			
-Admission	5.78 ±1.74	5.96 ±2.06	0.820[3]
-Discharge	5.39 ±1.72	6.28 ±2.07	0.254[3]
Length/height			
-Admission	61.50 ±7.19	63.96 ±8.60	0.443[3]
-Discharge	61.72 ±7.29	64.54 ±8.38	0.373[3]
STRONGkids at admission, n (%)	3.22 ±0.66	2.82 ±0.67	0.127[3]
-Moderate risk	6 (66.67)	0.3732	
-High risk	3 (33.33)	5 (17.86)	0.373[2]
STRONGkids at discharge, n (%)	3.56 ±0.88	2.82 ±0.66	0.012[3]
-Moderate risk	4 (44.44)	0.0412	
-High risk	5 (55.56)	5 (17.86)	0.041[2]
Albumin levels, g/dL			
-Admission	3.57 ±0.54	3.45 ±0.55	0.579[3]
-Dsciharge	3.05 ±0.45	3.21 ±0.46	0.356[3]
-Change (Δ)	-0.52 ±0.57	-0.24 ±0.53	0.631[3]
TLC, cells/mm3			
-Admission	3,996.67 ±2,668.03	3,707.14 ±1,784.06	0.711[3]
-Discharge	3,006.66 ±2,173.74	3,077.14 ±1,293.78	0.906[3]
-Change (Δ)	-990.00 ±2,528.15	-630.00 ±1,727.02	0.631[3]

[1]Independent sample T-test, [2]Fischer exact test, [3]Mann-Whitney U test, [4]Pearson Chi-square test

The subjects were divided into two groups on the basis of body weight loss since admission: the hospital malnutrition group (abbreviated as HMN, n=9) and the non-hospital malnutrition group (abbreviated as non-HMN, n=28). Other than the STRONGkids scores and other parameters at admission, there were no noticeable differences between the two groups. The hospital malnutrition group had a higher risk (55.56%) compared to the non-hospital malnutrition group, as indicated by the STRONGkids scores of 3.56±0.88 and 2.82±0.66, respectively (p=0.012).

Table 2. Correlation of the STRONGkids and hospital malnutrition with the biochemical markers.

Variable	STRONGkids score at admission	Body weight change → HMN ruler
Length of hospital stay (LOS)	r=0.413, p=0.011[2]	r=0.283, p=0.089[1]
Body weight at discharge	r=-0.434, p=0.007[2]	r=0.013, p=0.941[1]
Albumin at discharge	r=-0.521, p=0.001[2]	r=0.030, p=0.862[1]
Delta (Δ) Albumin	r=0.193, p=0.251[2]	r=-0.033, p=0.647[1]
TLC at admission	r=-0.475, p=0.003[2]	r=0.028, p=0.868[1]
TLC at discharge	r=-0.665, p=0.000[2]	r=-0.097, p=0.862[1]
Delta (Δ) TLC	r=0.096, p=0.572[2]	r=-0.112, p=0.510[1]

[1]Pearson's correlation, [2]Spearman's rho

Table 2 shows the correlation of STRONGkids scores and hospital malnutrition with TLC and serum albumin. The STRONGkids scores were negatively correlated with body weight at discharge (r=-0.434, p=0.007), serum albumin at discharge (r=-0.521, p=0.001), and TLC at admission (r=-0.475, p=0.003) and at discharge (r=-0.665, p=0.000). There was no other significant difference between the variables (p>0.05). The hospital malnutrition did not show any correlation with the other parameters.

Table 3. Bivariate analysis of the biochemical markers associated with the STRONGkids score.

	Hypoalbuminemia at discharge	Low TLC at discharge
B	1.082	1.714
SE	0.862	1.723
Exp (B)	2.951	5.549
p[1]	0.209	0.320
95% CI		
Lower	0.545	0.190
Upper	15.980	162.360

As shown in Table 3, hospital malnutrition increased the TLC by 5.549 times (95% CI [0.190-162.360]) and the risk of hypoalbuminemia by 2.951 times (95% CI [0.545-15.980]) at discharge.

Table 4. Bivariate analysis of the biochemical markers associated with hospital malnutrition.

	Hypoalbuminemia at discharge	Low TLC at discharge
B	1.872	2.104
SE	0.988	1.760
Exp (B)	6.500	8.196
p[1]	0.058	0.232
95% CI		
Lower	0.937	0.260
Upper	45.103	258.206

Table 4 demonstrates that a high-risk STRONGkids score also increased the risk of hypoalbuminemia at discharge by 6.5 times (95% CI [0.937-45.103], p=0.058) and of low TLC by 8.196 times (95% CI [0.260-258.206], p=0.23).

DISCUSSION

In the absence of appropriate perioperative nutritional support, the incidence of malnutrition in hospitals is associated with longer hospital stays, complications, and an increased risk of mortality (Mosquera et al. 2016). In this study, there was a correlation between hospital malnutrition and STRONGkids score, but not with the other factors examined. However, with the aid of a nutritional risk screening tool and biomarker parameters, the risk of hospital malnutrition could be identified earlier, thereby preventing patients from developing additional disease-related complications (Mehta & Compher 2009, Rocha & Fortes 2015, Thibault et al. 2021).

STRONGkids is a valid questionnaire that is used as a screening tool for malnutrition in various parts of the world (Carter et al. 2020). STRONGkids was used as the nutritional risk screening tool in this study not only due to the hospital's guidelines, but also because a NewZealand study demonstrated that it was practical and easier to use than other tools for identifying children at risk of malnutrition (Moeeni et al. 2014). STRONGkids is a useful tool for identifying the risk of severe malnutrition in hospitalized patients (Malekiantaghi et al. 2022). Hulst et al. (2020), conducted a multi-center study in hospitals and STRONGkids. They discovered a link between having a "high risk" STRONGkids score and having a negative SD-score in weight-for-height. Another systematicreview study demonstrated that STRONGkids was a valid tool for detecting

hospital malnutrition risk (dos Santos et al. 2019). STRONGkids can detect malnutrition earlier than anthropometric measures and attend to patients' nutrition changes due to hospitalization, so they can be useful tools for hospitalized children.

The STRONGkids scores were analyzed to determine whether there was any significant difference between the hospital malnutrition and non-hospital malnutrition groups at admission and discharge. The STRONGkids scores of the hospital malnutrition and non-hospital malnutrition groups differed significantly, indicating that STRONGkids and hospital malnutrition were correlated. This is explained by the STRONGkids employed to identify hospital malnutrition in the patients, with the assessed factors including subjective clinical evaluation, high-risk diseases, reduced nutritional intake, weight reduction or poor weight gain (Figure 2). Referring to Figure 3, the STRONGkids scores are totaled based on the aforementioned factors and then categorized as high-, medium-, or low-risk. If a patient's score is in the moderate- or high-risk categories, hospital malnutrition is most likely present.

The STRONGkids scores at discharge between the hospital malnutrition and non-hospital malnutrition groups demonstrated a significant difference in this study (p=0.012). The scores serve to describe the current nutritional state, the frequency of weight loss, and a decrease in food ingestion, all of which contribute to the tendency of weight loss (Tommy et al. 2022). To track any weight changes that could have an impact on the patient's condition, the scores were evaluated twice, once before surgery and once after, in accordance with the hospital policy (Figure 1). Despite being correlated with the STRONGkids scores at discharge, the scores at admission did not significantly differ between the groups, which we assume was due to the amount of data available from medical records.

There have been research examining the relationship between the length of hospital stay and STRONGkids score since hospital malnutrition is frequently associated with hospital stays. A univariate analysis demonstrated that children with low-risk scores had noticeably shorter length of stay compared to those with medium- or high-risk scores, with the median of two versus three days between the low risk and medium- or high-risk scores, respectively (p<0.001). A multivariate study also demonstrated that the nutritional risk category continued to be important in demonstrating length of hospital stay discrepancies between low-risk and medium- or high-risk even if other factors linked to prolonged hospitalization were considered (Hulst et al. 2010).

The STRONGkids and biochemical parameters

This study investigated whether the correlation of STRONGkids with serum albumin and TLC had an impact on the likelihood of hospital malnutrition. It was discovered that serum albumin levels measured at discharge was related to the STRONGkids scores, but not to hospital malnutrition. In other words, the lower the serum albumin level, the higher the STRONGkids score.

Even though there has not been any research on the specific connection between serum albumin and hospital malnutrition, the relationship between low serum albumin levels and the STRONGkids score can be explained by acute stress in the body that led to the decrease of serum albumin level. Acute stress-induced inflammation can result in capillary leaks that may cause the extravasation of serum proteins into the interstitial space, reduced oncotic pressure that initiates extravasation within the inflammatory state, intravascular volume depletion, and loss of serum proteins (Loftus et al. 2019). This would imply a link between serum albumin and hospital malnutrition, albeit indirectly, through the recognized evidence of how STRONGkids can be deemed a helpful and valid measurement of nutritional risk (dos Santos et al. 2019). Another study focusing on adolescents and children with liver disease used the STRONGkids in the nutritional risk assessment to examine the relationship between serum albumin and dietary intake. They discovered that more patients in the high-risk group had lower serum albumin and prealbumin than those in the moderate-risk group (p<0.001) (Song et al.2017).

This study demonstrated a correlation between the STRONGkids scores and TLC at admission and discharge, which implying that the greater the STRONGkids score, the higher the malnutrition risk. This occurence was explained by how low TLC could degrade lymphocyte quality, making children more susceptible to recurring infections and inflammation, which changed the metabolism process (including the energy, protein, and mineral metabolism) within the infected individual. Following this condition, energy requirements would increase to aid in the elimination of the infection process, as well as the depletion of glycogen and fat reserves. Malnutrition will occur if immediate nutritional supplementation is not provided (Ibrahim et al. 2017). In light of this, a recent study from Sanglah General Hospital, Bali, Indonesia, revealed a significant correlation between TLC and the occurrenceof hospital malnutrition (p=0.002). Using a cut-off value of 4,000 cells/mm^3, children between the ages of 6 and 12 months were found to have a 61.8% hospital malnutrition incidence rate and a 32.3% rate of normal TLC, as well as a 3.9 times

higher risk of hospital malnutrition for those with low TLC, with a 95% confidence interval between 1.5 and 7.1. These data suggested that low TLC levels can be used to predict the development of hospital malnutrition (Ekaputri et al. 2021).

Other nutritional screening tools

STRONGKids exhibited the highest specificity (100%) to detect acute malnutrition in hospitalized children, with a positive predictive value of 36% when compared to the Screening Tool for the Assessment of Malnutrition in Paediatrics (STAMP), Pediatric Yorkhill Malnutrition Score (PYM), and Finnish national growth charts (Tuokkola et al. 2019). However, because there has not been a study that estimates the risk of low albumin and low TLC in child patients, the study examined the sensitivity of the STRONGkids based on anthropometric measurements rather than biochemical markers of malnutrition, e.g., albumin and TLC.

A study in Yogyakarta,Indonesia, using the Simple Nutrition Screening Tool (SNST), Nutritional Risk Screening (NRS) 2002,Malnutrition Screening Tool (MST), Malnutrition Universal Screening Tool (MUST), and Short Nutritional Assessment Questionnaire (SNAQ) revealed that medium- and high-risk malnourished patients had low albumin levels that were 2.84-2.98 times higher in elderly (Susetyowati et al. 2018). Another study found that the elderly, particularly those with a lower level of education, frequently consume an insufficient amount of energy (Setiati et al. 2013). Robinson (2015) also discovered that older people with low serum albumin had a 3.27 hazard ratio for hospital readmission within 30 days. Mild hypoalbuminemia in colorectal cancer patients increased the risk of mortality by 1.74 times (Hu et al. 2019). Patients who underwent abdominal surgery and had a nutritional risk index (NRI) score below 97.5 were 1.8 times more likely to experience poor wound healing (Hussen et al. 2020). The likelihood of low TLC in COVID-19 patients was also 3.05 times higher when malnutrition was present (Zhang et al. 2022)

Strength and limitations

As there were few studies in Indonesia that discussed hospital malnutrition in children admitted to surgery and its risk screening, this study's strength was that it offered useful information about the incidence of hospital malnutrition and the STRONGkids' use among pediatric patients who were scheduled for surgery. It will also help future studies to investigate more about serum albumin and TLC and their correlation with the occurrence of hospital malnutrition.

One of the study's limitations was that the subjective assessments of the STRONGkids were performed by many clinicians, as the data were all gathered from medical records. It also did not allow this study to accurately monitor a wider range of the patients' condition. Another limitation of this study that might have resulted in insignificant findings between hospital malnutrition and other parameters was that the data being analyzed with an insufficient number of accessible medical records and time inconsistency while retrieving the biochemical markers. We suggest future studies to conduct prospective study and observe additional patient outcomes (i.e., secondary infections and presence of edema).

CONCLUSION

This study proved that total lymphocyte count and serum albumin cannot be utilized as independent markers of hospital malnutrition. However, with the use of a nutritional risk screening tool (i.e., STRONGkids), total lymphocyte count and serum albumin can both operate as additional markers to help identify the risk of hospital malnutrition to avert complications in children undergoing gastro-intestinal surgery.

Acknowledgment

The authors would like to acknowledge the reviewers' constructive feedback.

Conflict of interest

None.

Ethical consideration

An ethical approval was obtained from the Research Ethics Commission, Dr. Soetomo General Academic Hospital (No. 0653/LOE/301.4.2/X/2021 on 18/10/2021).

Funding disclosure

None.

Author contribution

HAR oversaw the conception or design of this study, as well as the data acquisition, analysis, and interpretation. NAW, RI, and AS critically examined and revised the manuscript for important intellectual content and approved the final version for publication.

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Original Research Report

ANALYSIS OF SPATIAL WORKING MEMORY USING THE Y-MAZE ON RODENTS TREATED WITH HIGH-CALORIE DIET AND MODERATE-INTENSITY EXERCISE

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ABSTRACT

Spatial working memory (SWM) in humans and animal models with impaired cognitive functions has been analyzed through a number of methods. However, this is still understudied in animal models treated with a high-calorie diet (HCD) and moderate-intensity exercise (MIE). The Y-maze was utilized as the assessment method in this study. A 40 x 9 x 9 cm^3 Y-maze was employed to observe the animal models' spontaneous alternation (SA) as the representation of their SWM. This was done by calculating the total alternation percentage divided by total entry minus two. A total of 17 female *Mus musculus* mice aged 8 weeks were tested in the Y-maze to investigate their SWM using SA calculations. Each mouse was analyzed for eight minutes and recorded in a dark and quiet room to minimize bias due to environmental noise and lighting. Comparing the treatment group's (HCD+MIE) SA to the control group's SA revealed no statistically significant difference ($p=0.451$). Seven mice in the treatment group performed similarly to the mice in the control group in the Y-maze test, with no significant difference in their ability to complete the task. The mice in the treatment group exhibited no motor impairment, as indicated by complete movements of all their extremities while exploring the Y-maze within the allotted time. In conclusion, the Y-maze can be used as a reliable method to analyze SWM in overweight/obese *Mus musculus* animal models treated with moderate-intensity physical exercise.

Keywords: Spatial working memory; Y-maze; cognition; mice; obesity

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Article history
●Submitted 22 Dec 2022 ● Revised 27 Jan 2023 ● Accepted 10 Feb 2023 ● Published 10 Mar 2023

How to cite: Melbiarta PP, Kalanjati VP, Herawati L, et al (2023). Analysis of spatial working memory using the Y-maze on rodents treated with high-calorie diet and moderate-intensity exercise. Folia Medica Indonesiana, 59 (1), 40-45. https://doi.org/10.20473/fmi.v59i1.32672

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. This article reports the use of the Y-maze as a simple yet effective method to measure spatial working memory in mice.
2. The Y-maze method can be used safely without exposing the animals to additional stressors, as evidenced by the absence of mortality following the test.

INTRODUCTION

The global death and disability-adjusted life years attributable to obesity have increased, indicating that it continues to be a major health concern (Dai et al. 2020). Obesity caused by a high-calorie diet (HCD) has been associated with early cognitive decline (i.e., early-onset Alzheimer's disease with unclear pathophysiology). Neurodegeneration processes, including neuroinflammation, synaptic disruption, and neuronal cell death have been observed to be similar to those observed in Alzheimer's disease (AD) patients who are typically over 65 years old (Lam et al. 2013, Tellechea et al. 2018, Mendez

2019, Eugenia et al. 2022). Reportedly, certain physical exercise regimens, including moderate-intensity exercise (MIE), aid in the recovery of impaired tissue and cells caused by HCD-related obesity (Dye et al. 2017, Tellechea et al. 2018, Coll-Padrós et al. 2019). Physical exercise in general also has a positive influence on subjects with stroke and cognitive function impairment (Aisyah et al. 2020).

Spatial working memory (SWM) is a type of neurocognitive function that correlates with the hippocampal and prefrontal cortex nerve pathways. Neuronal proliferation and apoptosis have been reported to be significantly affected by exercise in obese rodents (Laing et al. 2016, Wirt & Hyman 2017). Overweight and obese individuals have been reported to suffer from cognitive impairment with varying degrees and morphological changes related to cognitive function (Cook et al. 2017, Sack et al. 2017, Meo et al. 2019). In general, HCD-treated animal models exhibited the pathophysiology of overweight and obesity. It has been reported that moderate-intensity exercise (MIE) mitigated the adverse effects of HCD, and that it improved cognitive functions (Rebelo et al. 2020, de Sousa et al. 2021). Several studies reported the use of Y-maze as a tool to analyze the SWM in various animal models (Woo et al. 2018, Faradila et al. 2020). The aim of this study was to investigate if the Y-maze is reliable as a tool to measure SWM in overweight and/ or obese animal models treated with MIE, since this has not been widely explored in a lot of other studies.

MATERIALS AND METHODS

Seventeen female *Mus musculus* mice, aged 8 weeks, were tested using the Y-maze (Figure 1) to study the SWM using the spontaneous alternation (SA) calculation. Each mouse was analyzed for eight minutes and recorded in a dark and quiet room to reduce the influence of environmental noise and lighting on the nocturnal animals. The SA of the treatment group (HCD+MIE) (n=7) was then compared to those of the control group (n=10). Similar to a study by Herawati et al. (2020) and Kumalasari et al. (2021), the treatment group received 0.013 g/g body weight of 40% dextrose (D40) as the HCD. As suggested by Rahayu et al. (2021), the MIE for the treatment group consisted of swimming three times per week with a 6% body weight tail-attached load, and the duration was increased every week (5 m in week-1, 8 m in week-2, 11 m in week-3, 15 m in week-4). The body weight (g) of each mouse was measured using a digital scale (Idealife, Indonesia) prior to the experiment and after four weeks of treatment.

The Y-maze used was a black triangular maze made of polyvinyl chloride (PVC). As shown in Figure 1, the maze consisted of three arms (A, B, and C), each measuring 9 cm in width, 9 cm in height, and 40 cm in length. Prior to the experimentation, the mice were acclimatized in the laboratory for at least seven days. The Y-maze had to be cleaned using 70% alcohol and paper towels to ensure the absence of an odorous smell that might interfere with the experiment. After the maze was completely dry, the camera (Oppo, China) was placed above the maze in a way that all of its arms were fully recorded.

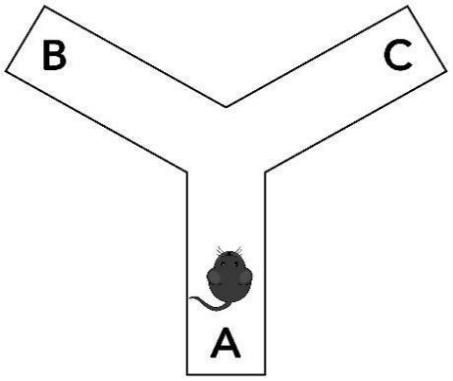

Figure 1. The Y-maze used in this study, with a dimension of 40 x 9 x 9 cm^3 and 120^o between the two legs, made of polyvinyl chloride (PVC) and assembled using PVC glue.

The experiment was conducted in an environment that simulated the nighttime. All materials were prepared before the experimentation. After the Y-maze settings had been completed, the mice were placed on arm A. The recording began simultaneously with the placement of the mice. The mice were allowed to move freely in all of the arms for 8 minutes. Afterwards, the recording was stopped and the mice were taken out from the maze. The maze should also be wiped down with 70% alcohol and paper towels after each task (Kraeuter et al 2019). The total entry and total alternation were counted from the recordings. Each time all four legs of the mouse completely entered an arm of the Y-maze, it was considered an entry. If the mouse could enter all three arms (A, B, and C) consecutively, it was considered an alternation. Spontaneous alternation (SA) is an indicator of spatial working memory that is calculated by dividing actual alternation (total alternation) by total alternation possibilities (total entry minus two) multiplied by 100 percent, as shown in the equation (Kraeuter et al. 2019).

$$Spontaneous\ alternation = \frac{Total\ alternation}{Total\ entry - 2} \times 100\%$$

The data from the observation were presented as mean±SD. Normality test and homogeneity test using Shapiro-Wilk and Levene's tests, respectively, were performed prior to the independent t-test to compare the SA between the two groups, where a p-value<0.05 was considered significant. The statistical analysis was performed with the help of SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, Ill., USA).

RESULTS

Table 1 displays the characteristics of each group's body weight. Although not statistically significant (p=0.957), the treatment group had a higher body weight than the control group prior to the experiment. After four weeks, the average body weight of the treatment group was lower when compared to those of the control group (p=0.379).

Table 1. Average body weight of treatment and control groups showed paradoxical modulation during the four-week experiment.

	Mean±SD		
Body weight	Control group (n=10)	Treatment group (n=7)	p-value
Pre (g)	23.29±0.81	24.00±0.95	0.957
Post (g)	23.80±0.74	22.75±1.18	0.379

Table 2. Comparison of spontaneous alternation (SA) between control and treatment groups.

Subject	Spontaneous Alternation (%)	
	Control Group (n=10)	Treatment Group (n=7)
Animal 1	60.92	62.04
Animal 2	68.29	70.59
Animal 3	73.68	56.21
Animal 4	67.27	67.9
Animal 5	51.25	71.43
Animal 6	55.81	64.52
Animal 7	66.23	54.1
Animal 8	79.31	-
Animal 9	53.85	-
Animal 10	66.67	-
Mean±SD	64.33±8.89	63.83±6.79
p-value between the groups'standard deviation (SD)	0.451	

Seven mice in the treatment group performed in the Y-maze test with results comparable to those of the control group. All animals showed no significant difference in their ability to complete the task. Comparing the treatment group to the control group, no indication of motor impairment was observed. All extremities of the mice exhibited complete movements when exploring the arms of the Y-maze in the allotted time.

The mean of the SA was 63.83±6.79 (%) in the treatment group and 64.33±8.89 (%) in the control group. The values of the treatment and control groups' SA were not significantly different (p=0.451), as shown in Table 2.

DISCUSSION

This study reveals that the treatment group experienced a decrease in body weight, while the control group exhibited a contrast pattern. Although the difference in body weight between these two groups before and after the experiment was not statistically significant, the decrease pattern in the treatment group might indicate a response to the physical stressors compared to the control group. In overweight and obese animal models, such as those treated with HCD, physical exercise may improve altered metabolic and cognitive functions. A study on rodents treated with a high-glucose diet and physical exercises reported improved long-term memory and glucose tolerance. Other research reported that the brain cortical capillary volume and surface area of rodents with a high-fructose diet and physical exercise were significantly greater than those of the control group (Wang et al. 2015, Rebelo et al. 2020).

By calculating the SA in animal models treated with HCD and MIE, the Y-maze proved to be a reliable tool for measuring SWM in this study. Comparable SA between treated and control groups demonstrated this. MIE can exert neuroprotective properties by preserving cellular function, possessing anti-inflammatory properties, and enhancing the release of neurotrophic factor, although this may be influenced by the type, duration, and intensity of the physical exercise (Kim et al. 2019, Cerqueira et al. 2020).

Dopamine plays an important role in the modulation of spatial working memory. Environmental stimulation induces the firing rate of dopaminergic neurons in the ventral tegmental area, resulting in dopamine release (Bäckman et al. 2017). Increased dopamine will activate neurons in the prefrontal cortex. These neurons will process the information collected from the cortex in the form of working memory. Several forms of dopamine exist, including dopamine-1 (D1) and dopamine-2 (D2), which have dynamics related to the decision-making process. The activation of D1 receptors will be enhanced with Ca^{2+}, K^{+}, and Na^{+}, while the activation of D2 receptors decreases with L-type Ca^{2+} through induction after depolarization. This mechanism was found to be the basis of spatial working memory, in which hippocampus-processed spatial memory undergoes additional analysis to form new memories

(Puig et al. 2014).

There are various methods to assess the working memory in mice other than the Y-maze, such as the Morris water maze (Maramis et al. 2021). Xu et al. (2015) reported the use of the Morris water maze to study spatial learning and memory in HCD-induced obese mice. Another study by Tian et al. (2017) reported the use of T-maze in post-surgery mice as a simple behavioral task to assess spatial working memory. The Y-maze had been utilized as a method that can be constructed by researchers with simple materials, but when used correctly, could be used to analyze spatial working memory. The interpretation of spontaneous alternation as an indicator of spatial working memory is based on the instinct of a mouse to explore new territory. A higher alternation represented superior performance in spatial working memory as it implied that the mouse remembered the arm it entered (Heredia-López et al. 2016, Kraeuter et al. 2019).

Strength and limitations

In this study, other biomarkers from the research animals were not analyzed. However, this study can help to determine the Y-maze as a tool for measuring SWM in overweight and/ or obese animal models treated with MIE, which has not been fully reported previously.

CONCLUSION

Y-maze can be used to analyze the spatial working memory in *Mus musculus* as the animal models in an experiment. More precisely, this model has proven effective in experiments in which physical exercise is applied to treat overweight and/ or obese *Mus musculus*. The Y-maze method offers a simple and effective, yet safe way of studying this variable, thus helping further studies in elucidating neurocognitive impairment prevention and management.

Acknowledgment

The authors thank the Faculty of Medicine, and Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

Conflict of interest

None.

Ethical consideration

Ethical clearance of this study was granted by the Health Research Ethics Committee, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia (no. 259/EC/KEPK/FKUA/2021 on 22/11/2021).

Funding disclosure

This study was funded by the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. The benefactors had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contribution

RRM collected, analyzed, and interpreted the data, as well as drafted, revised, and approved the final manuscript. VPK conceptualized the study design, analyzed and interpreted the data, as well as drafted, revised, and approved the final manuscript. LH conceptualized the study design, interpreted the data, as well as revised and approved the final manuscript. YS drafted, revised, and approved the final manuscript. ZO checked and approved the final manuscript.

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Original Research Report

PROPORTIONS OF GROUP B *Streptococcus* ISOLATION FROM PREGNANT WOMEN'S VAGINAL AND RECTAL SWAB SPECIMENS AT A TERTIARY HOSPITAL IN SURABAYA, INDONESIA

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ABSTRACT

Group B *Streptococcus* is a Gram-positive bacterium found in women. It causes high-risk mortality in pregnant women, newborns, and the elderly. This study aimed to compare group B *Streptococcus* (GBS/*Streptococcus agalactiae*) proportions from different collection sites (vaginal and rectal swabs). This was an analytic observational study with a hospital-based cross-sectional design. A total of 74 swabs were taken from 37 pregnant women at 35–37 weeks of gestation. Each participant provided a vaginal swab and a rectal swab, which were cultured in Todd Hewitt broth, blood agar, and CHROMagar. The specimens were subsequently identified using the VITEK 2 system. The GBS isolation percentages from the vaginal and rectal swab specimens were determined to be 13.5% and 8.1%, respectively. The McNemar test had a result of 0.697, and the Cohen's kappa test had a result of 0.165. To conclude, there was no significant difference in GBS isolation proportions between the vaginal and rectal swab cultures. Combined vaginal and rectal swab cultures were required to increase GBS isolation from pregnant women.

Keywords: Proportion of *Streptococcus agalactiae*; vaginal swab; rectal swab; maternal health

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Article history
●Submitted 7 Dec 2022 ● Revised 17 Jan 2023 ● Accepted 12 Feb 2023 ● Published 10 Mar 2023

How to cite: Ivanna, Wasito EB, Debora K. (2023). Proportions of group B *Streptococcus* isolation from pregnant women's vaginal and rectal swab specimens at a tertiary hospital in Surabaya, Indonesia. Folia Medica Indonesiana, 59 (1), 46-50. https://doi.org/10.20473/fmi.v59i1.11708

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. Rectal and vaginal swab specimens were collected from pregnant women, and there was no significant difference in the proportions of group B *Streptococcus* isolation.
2. Combined vaginal and rectal swab cultures provide higher isolation of group B *Streptococcus*.

INTRODUCTION

Group B *Streptococcus* (GBS/*Streptococcus agalactiae*) is an encapsulated, betahemolytic, catalase-negative, and facultative anaerobe coccus found in the human commensal microbiome. The source of vaginal GBS colonization in women is the gastrointestinal (GI) tract (Hanson et al. 2022, Wang et al. 2022). Group B *Streptococcus* is still a major cause of morbidity and mortality in high-risk populations, such as pregnant women, newborns, and the elderly. It can cause preterm births, fetal injury, premature membrane ruptures, fetus infection, sepsis, meningitis in infants, and fetal demise (Raabe & Shane 2019, Kurian & Modi 2022, Suwardewa et al. 2022).

GBS is a Gram-positive bacterium found in 5-30% of women's vaginal and gastrointestinal tracts (Tille 2014). Rectovaginal colonization by GBS occurs in 10 to 30% of pregnant women and is responsible for many perinatal and neonatal infections (Szymusik et al. 2014). Currently, data on GBS colonization and invasive bacterial disease in the Indonesian population are limited. A study conducted in Denpasar, Bali, Indonesia, between 2007–2008 reported that the GBS colonization rate was 31.3% among 32 pregnant women with gestational ages of 35–37 weeks (Sri-Budayanti & Hariyasa-Sanjaya 2013). In Jakarta, Indonesia, the GBS colonization

rate in pregnant women was 30% (53 out of 177). These rates were higher than the other Asian countries mean rate of 12.8% (country variation: 8%-20%) and also higher than the global rate of GBS colonization (Russell et al. 2017, Edwards et al. 2019, Safari et al. 2021).

Important knowledge to prevent diseases in high-risk pregnancies should be acquired through screenings, such as the Poedji Rochjati Score Card (Simanungkalit et al. 2021). The Centers for Disease Control and Prevention (CDC) recommended a universal antenatal culture-based screening at 35-37 weeks of gestation. Screenings are crucial for identifying bacteria with high resistance to antibiotics (Linggarjati et al. 2021, Sulikah et al. 2022), and also preventing other conditions that may pose a risk to reproductive health (Hanifah et al. 2018, Kurniawati et al. 2019). The recommendation also suggested rectovaginal specimen collection in order to obtain an adequate yield of GBS (Kwatra et al. 2013). The accuracy of colonization status can be enhanced by improving culture timing, adding more specimen collection locations, and utilizing the correct culture and detection methods (Kwatra et al. 2013).

Rectal swabs may provide a quick and convenient method for analyzing the colonic microbiome. Rectal swabs obtained from clinicians are a reliable method of analyzing the colonic microbiome. Because antibiotics influence the microbiome, obtaining specimens for microbiome analysis is often time-critical. Rectal swabs are demonstrated to be a valid and practical method for microbiome analysis (Turner et al. 2022).

Although there were numerous studies on GBS, the results of some studies concerning the site of specimen collection were inconclusive. Rosa-Fraile & Spellerberg (2017) reported that rectovaginal swabs were more likely to yield positive cultures than vaginal swabs only (100% versus 50%, respectively). Khalil et al. (2017) reported that rectovaginal specimens had a lower detection rate than vaginal and rectal specimens. Nadeau et al. (2022) reported similar results of GBS-positive rectal and vaginal swab specimen cultures. In a study conducted by Bidgani et al. (2016), rectal swabs yielded more positive cultures than vaginal swabs. Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, did not have universal culture-based screening at 35-37 weeks of gestation at the time this study was conducted. Therefore, the authors compared GBS culture detection rates in pregnant women from different sample collection sites (vaginal and rectal swabs).

MATERIALS AND METHODS

This research was an analytic observational study with a hospital-based cross-sectional design. It was conducted at the Department of Obstetrics and Gynecology and the Microbiology Laboratory of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, since February until April 2018. As previously used in a study by Bidgani et al. (2016), the sample inclusion criteria of this study were vaginal and rectal swab specimens from pregnant women between 35 and 37 weeks of gestation. The technique used for collecting the samples was consecutive sampling.

Vaginal and rectal swabs were collected from each subject, then the specimens were inoculated within 24 hours in a selective broth culture medium, i.e., Todd Hewitt broth. The broth was incubated for 18 to 24 hours at 35˚ C and subcultured on blood agar plates and CHROMagar plates for 24 hours, as suggested by Kwatra et al. (2013). The data were analyzed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, New York, USA), with a 95% confidence interval and a significance (p-value) ≤0.05. The McNemar test was used for two related measurements on the same sample or when each individual measurement in one sample could be paired with a specific measurement in the other sample. The McNemar test for paired proportions was utilized to examine the relationship between the two specimen collection methods and the test results of the specimens. Agreements between pair of cultured methods were assessed by Cohen's kappa statistic. Cohen's kappa statistic was used to assess the concordance between the two culture methods.

RESULTS

During the study period, 74 swabs (37 vaginal swabs and 37 rectal swabs) were obtained from 37 pregnant women who met the inclusion criteria. Seven of 37 pregnant women indicated GBS colonization from at least one collection site. The prevalence of intrapartum GBS colonization among pregnant women at Dr. Soetomo Hospital was 18.9%. The detection rate of GBS was higher in the vaginal swab specimen culture than the rectal swab specimen culture. The proportions of GBS collected from the vaginal and rectal swabs were 5 (13.5%) and 3 (8.1%), respectively.

Table 1. Comparison of GBS cultures from pregnant women's vaginal and rectal swab specimens.

	Vaginal culture		Total
	Positive	Negative	
Rectal culture			
Positive	1	2	3
Negative	4	30	34
McNemar p-value	0.687		
Kappa value	0.165		
Total	5	32	37

The detection rates of GBS did not differ significantly between the vaginal and rectal swab methods (Table 1). The p-value from the McNemar test was 0.687, while the Kappa value was 0.165 for GBS detection in the vaginal and rectal cultures.

DISCUSSION

Group B streptococcus is a leading cause of neonatal bacterial sepsis and meningitis. The risk of colonization in newborns rises if the mother is heavily colonized with this bacterium (Bigdani 2016). In most populations studied, 10–30% of pregnant women were colonized with GBS in the vaginal or rectal area. Similarly, this study discovered that 18.9% of pregnant women were colonized by GBS. Both anatomic site sampling and culture methods are important in maximizing GBS carriage detection rates. Rectovaginal swabs have been reported to provide high bacterial yields, as the gastrointestinal tract is a natural reservoir for GBS and a potential source of vaginal colonization (Bigdani 2016).

In this study, a non-significantly higher GBS detection rate was observed in the vaginal region than in the rectal region (13.5% vs 8.1%), with p=0.687 in the McNemar test. Other studies have also reported a slightly higher detection rate in vaginal swab specimen cultures compared to rectal swab specimen cultures (Africa & Kaambo 2018). However, in Russell et al. (2017) reported a higher GBS detection rate in rectal swab cultures than in vaginal swab cultures (18% vs 24%), as did Bidgani et al. (2016) (17.9% vs 10.2%).

The Cohen's kappa statistic coefficient shows the inter-rater agreement in a study, with K>0.75 is considered as excellent agreement, 0.4<K<0.75 as good agreement, and 0<K<0.4 as poor agreement. The kappa coefficient for the detection of GBS from vaginal and rectal swab cultures was 0.165, indicating a poor agreement. The non-significant McNemar test results for the GBS detection from vaginal versus rectal swab cultures (p=0.687) indicated that both methods produced the same discrepancy. Therefore, a combination method was required to increase the GBS detection rate.

Rectovaginal swabs are recognized as the representative sampling technique for conducting culture in detecting GBS colonization, as these bacteria are part of the normal flora of the gastrointestinal tract and may be the source of vaginal colonization. Swabbing the lower vagina and rectum (through the anal sphincter) significantly improves the culture yield compared to sampling the cervix or vagina without also swabbing the rectum (Kwatra et al. 2013, Bidgani et al.2016). Gopal Rao et al. (2017) reported that the detection rate of GBS from rectovaginal swabs was significantly higher than from vaginal swabs or rectal swabs alone. Rosa-Fraile & Spellerberg (2017) reported that rectovaginal sampling provided positive culture more frequently than vaginal sampling Nadeau et al. (2022) described that a perianal culture could replace a rectal culture because the detection rate of GBS was comparable while women were spared the discomfort of a rectal culture.

Strength and limitations

This study can contribute data for future studies, especially in the proportions of group B *Streptococcus* (*Streptococcus agalactiae*) from different collection sites (vaginal and rectal swabs). The findings of this study may provide insight into the necessity of a combination of vaginal and rectal swab cultures in indicating the isolation of GBS from pregnant women. The limitations of this study were the lack of time and the small number of samples. However, this study's findings may still be used as preliminary data for future studies using large numbers of samples.

CONCLUSION

Combined vaginal and rectal swab cultures are required to increase group B *Streptococcus* (GBS) isolation among pregnant women. It provides more accurate results and a promised reduction of neonatal infection risks.

Acknowledgment

The authors would like to thank the Clinical Microbiology Department and Obstetrics and Gynecology Department of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, as well as everyone who supported the writing of this research article.

Conflict of interest

None.

Ethical consideration

This research was approved by the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, through the letter number 39/Pamke.KKE/I/2018 on 08/02/2018.

Funding disclosure

None.

Author contribution

All the authors contributed to the conceptualization, research design, data analysis, and interpretation of the obtained results. IV collected the specimens and wrote the manuscript.

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Original Research Report

EXPRESSIONS OF β-TRYPTASE AND CHYMASE IN LUNG MAST CELLS DUE TO ANAPHYLACTIC SHOCK THROUGH HISTOPATHOLOGICAL APPEARANCE AT DIFFERENT POST-MORTEM INTERVALS

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ABSTRACT

Anaphylactic shock is a hypersensitivity response, a commonly type I hypersensitivity involving immunoglobulin E (IgE). It is caused by an antigen-antibody reaction that occurs immediately after a sensitive antigen enters the circulation. Anaphylactic shock is a clinical manifestation of anaphylaxis that is distributive shock, characterized by hypotension due to sudden blood vessel vasodilation and accompanied by a collapse in blood circulation that can result in death. β-tryptase and mast cell chymase expressions in the lungs of histopathological specimens that had experienced anaphylactic shock were examined at different post-mortem intervals in this study. A completely randomized design (CRD) method was employed by collecting lung samples every three hours within 24 hours of death, and then preparing histopathological and immunohistochemical preparations. The mast cell tryptase and chymase expressions were counted and summed up in each field of view, and the average was calculated to represent each field of view. The univariate analysis yielded p-values of 0.008 at the 15-hour post-mortem interval, and 0.002 at the 12-hour post-mortem interval. It was concluded that tryptase and chymase can be utilized as markers of anaphylactic (non-anaphylactoid) shock in the lungs.

Keywords: Anaphylactic shock; β-tryptase; chymase; post-mortem interval; mortality

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Article history
●Submitted 26 Nov 2022 ● Revised 10 Jan 2023 ● Accepted 12 Feb 2023 ● Published 10 Mar 2023

How to cite: Putra BA, Susilo I, & Yudianto A (2023). Expressions of β-tryptase and chymase in lung mast cells due to anaphylactic shock through histopathological appearance at different post-mortem intervals. Folia Medica Indonesiana, 59 (1), 51-56. https://doi.org/10.20473/fmi.v59i1.40938

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. The post-mortem interval is related to tryptase and chymase expressions in anaphylactic shock incidence.
2. Forensic experts can utilize tryptase and chymase as markers of anaphylactic (non-anaphylactoid) shock that occurs in the lungs.

INTRODUCTION

Anaphylaxis is literally derived from the Greek words "*ana*" that means "return" and "*phylaxis*" that means "protection" (McLendon & Sternard 2022). The immune response that should protect against a specific disease, also known as prophylaxis, actually damages the tissue in the case of anaphylaxis. In other words, it does the opposite of what it should, which is why it is referred to as anti-phylaxis or anaphylaxis (Smith 2015). Anaphylaxis is a type of distributive shock that causes hypotension due to abrupt blood vessels vasodilation, followed by a collapse in blood circulation that can lead to death (Kounis et al. 2013). The clinical manifestation of anaphylaxis is anaphylactic shock (Poziomkowska-Gęsicka & Kurek 2020).

Anaphylactic shock is a hypersensitivity response, frequently type I hypersensitivity, involving immunoglobulin E (IgE). It is triggered by an antigen-antibody reaction shortly after a sensitive

antigen enters the circulation (Reber et al. 2017). Allergens or hazardous agents can cause type I hypersensitivity, which may be more dangerous in some people (Abbas et al. 2022). When asthmatic patients are exposed to these substances, it can cause IgE-mediated hyper-sensitivity that can be exacerbated by the presence of airway resistance (Yudhawati & Krisdanti 2019). In children with allergic rhinitis, house dust mite allergies can trigger anaphylactic episodes (Endaryanto & Nugraha 2022). People with medical disorders and medication dependence are required to receive immunotherapy, which may minimize the risk of anaphylactic shock (Shinee et al. 2019).

Anaphylactic shock has become more common every year. According to epidemiological data, there are 50-2,000 episodes per 100,000 patients (0.5-2%). The prevalence of anaphylactic shock in animals is still unknown (Shmuel & Cortes 2013). However, several cases of anaphylactic shock had been recorded in dogs (Bosmans et al. 2014) and calves (Choi et al. 2019) as a result of antibiotic medication. Non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics are the most common causes of anaphylaxis (Rengganis 2016). Post-mortem diagnosis of anaphylactic shock is a challenging task for pathologists today. The method for post-mortem examination of anaphylactic shock can use numerous mediators that play a role in its occurrence. However, it still needs development for its use in different situations.

MATERIALS AND METHODS

The study used a completely randomized design (CRD) approach to collect lung samples and determine the minimal sample size, as suggested by (Stefanski et al. 2018). The formula employed was $(n-1)(t-1)\geq 15$, which would require at least two rabbits if the result was $n\geq 2$. Histopathological and immunohistochemical preparations were conducted every three hours within the first 24 hours after death. The samples should be assessed quantitatively according to the modified Remmele method (Bayo 2019).

The 24-hour observation time and the observation organ (i.e., the lungs) were the independent variables in this study. Univariate analysis was used to determine the results in the form of statistical measures, tables, and graphs for each variable. Univariate analysis is used to examine each variable in the research findings (Canova et al. 2017). The purpose of univariate analysis is to summarize the measurement data set in such a way that the data set becomes usable information. The summary can be composed of statistics, tables, or graphs and is generated for each variable.

The dependent variables were the amount of immunoreactive mast cells and the immunohisto-chemical appearance of the lungs every three hours after death to assess detectable β-tryptase and chymase protein expressions in different post-mortem intervals (PMIs). These were the variables that resulted from the treatment and would be examined in this study. The control variables in this study were the species, sex, age, and body weight of the experimental animals, as well as the dose of the anaphylactic reaction agents and environmental conditions. As suggested by Hanlon & Vanderah (2010), the anaphylactic reaction agents were 1 mg of ovoalbumin and 0.5 ml of Freund's adjuvant.

RESULTS

A statistical analysis employing a multivariate repeated measure test revealed that there was an effect of the treatment on the expression of mast cell tryptase in the lungs, which was observed at different post-mortem intervals. Table 1 shows the expression of mast cell tryptase in the lungs of the experimental animals.

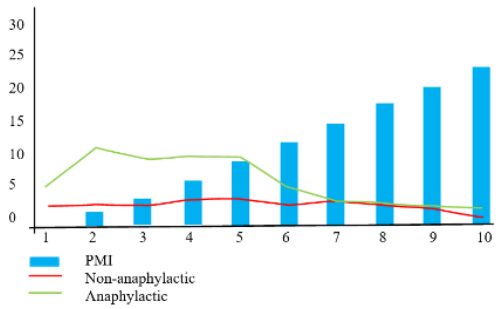

Figure 1. Lung mast cell tryptase of the anaphylactic and non-anaphylactic groups according to the PMIs.

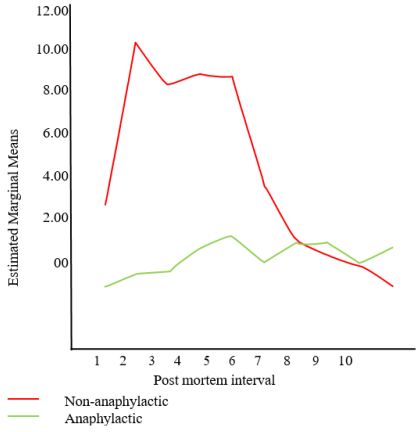

Figure 2. Interaction curve plots of the treatment groups with different PMIs.

Table 1. Data on mast cell tryptase in the lungs of the anaphylactic and non-anaphylactic groups.

PMI	Group	Average	SD
0 h	Non-anaphylactic	1.7500	1.06066
	Anaphylactic	4.8000	0.42426
	Total	3.2750	1.88038
1 h	Non-anaphylactic	2.2000	0.42426
	Anaphylactic	10.9000	0.28284
	Total	6.5500	5.03157
3 h	Non-anaphylactic	2.2500	1.060666
	Anaphylactic	9.2800	1.86676
	Total	5.7650	4.24385
6 h	Non-anaphylactic	3.1000	0.28284
	Anaphylactic	9.7000	1.69706
	Total	6.4000	3.93785
9 h	Non-anaphylactic	3.4500	0.07071
	Anaphylactic	9.6000	1.27279
	Total	6.5250	3.62618
12 h	Non-anaphylactic	2.4500	0.49497
	Anaphylactic	5.4700	0.38184
	Total	3.9600	1.78056
15 h	Non-anaphylactic	3.3000	0.14142
	Anaphylactic	3.3000	0.14142
	Total	3.3000	0.11547
18 h	Non-anaphylactic	2.7000	0.14142
	Anaphylactic	3.2000	0.00000
	Total	2.9500	0.30000
21 h	Non-anaphylactic	2.3000	0.28284
	Anaphylactic	2.3000	0.28284
	Total	2.3000	0.23094
24 h	Non-anaphylactic	1.6000	0.14142
	Anaphylactic	2.9000	0.00000
	Total	2.2500	0.75498

Observation at the 3-hour post-mortem interval showed that the quality of positive outcomes differed between the non-anaphylactic and anaphylactic groups. Mast cells, which were found in the submucosal connective tissue and interstitial tissue of the lungs, appeared to be proliferating in some fields of view. Tryptase expression was detected in the bronchioles and veins at the 3-hour post-mortem interval.

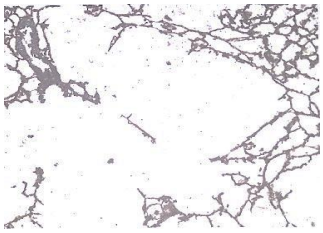

Figure 3. Immunohistochemical staining showed alveoli tissue in anaphylactic rabbits with emphysema (100x magnification of the lung mast cell tryptase).

At the 9-hour post-mortem interval, the non-anaphylactic and anaphylactic groups showed a difference in the quality of the positive results. There was a moderate accumulation of transudate fluid in the lumen of the alveoli and bronchioles in both the non-anaphylactic and anaphylactic groups. Bronchiolar integrity appears incomplete with negative immunoreactivity. The mast cell granules in the non-anaphylactic group were clearly visible intracytoplasmically.

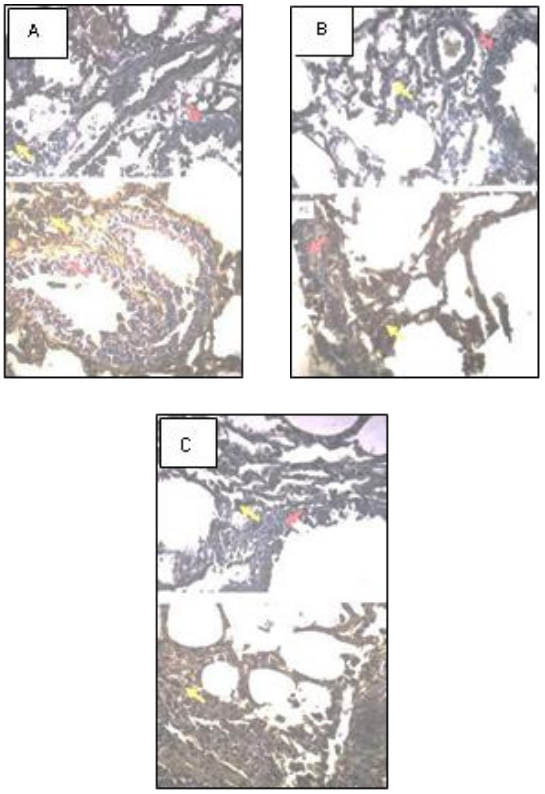

Figure 4. Immunohistochemical staining of mast cell tryptase at 0 h PMI (A), 1 h PMI (B), and 3 h PMI (C).

Red arrows in Figure 4 indicate negative immunoreactivity in the bronchioles. Yellow arrows indicate the mast cells. The anaphylactic group showed positive and proliferative immunoreactivity (400x magnification of the lung mast cell tryptase).

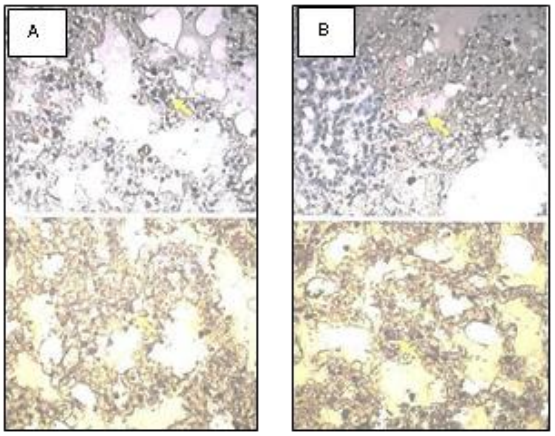

Figure 5. Immunohistochemical staining of mast cell tryptaseat 6 h PMI (A) and 9 h PMI (B).

Yellow arrows indicate the mast cells. The non-anaphylactic group showed positive immuno-reactivity in the mast cell granules (400x magnification of the lung mast cell tryptase.

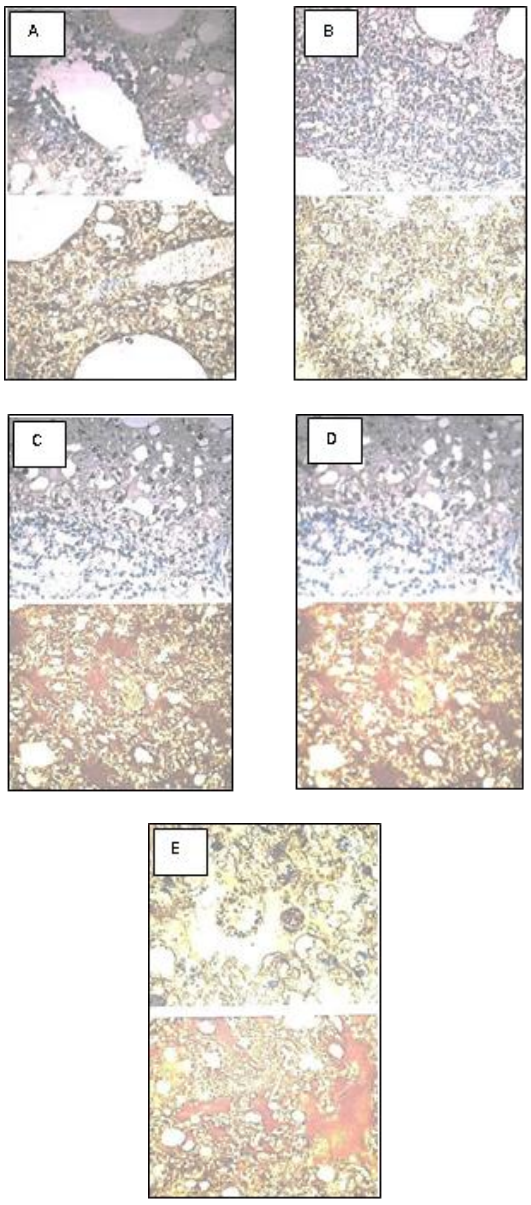

Figure 6. Immunohistochemical staining of mast cell tryptase at 12 h PMI (A), 15 h PMI (B), 18 h PMI (C), 21 h PMI (D), and 24 h PMI (E).

The bronchioles appeared damaged, and plasma had accumulated in the alveoli. The non-anaphylactic group showed weak positive immunoreactivity (400x magnification of the lung mast cell tryptase).

Table 2. Data on mast cell chymase in the lungs of the anaphylactic and non-anaphylactic groups.

PMI	Group	Average	SD
0 h	Non-anaphylactic	2.1500	0.77782
	Anaphylactic	5.0000	0.28284
	Total	3.5750	1.71343
1 h	Non-anaphylactic	2.6000	0.00000
	Anaphylactic	7.0500	1.34350
	Total	4.8250	2.68375
3 h	Non-anaphylactic	1.3500	0.07071
	Anaphylactic	7.2500	1.34350
	Total	4.3000	3.49380
6 h	Non-anaphylactic	2.4500	0.07071
	Anaphylactic	6.4300	0.52326
	Total	4.4400	2.31799
9 h	Non-anaphylactic	4.5600	1.04652
	Anaphylactic	6.4900	1.08894
	Total	5.5250	1.41491
12 h	Non-anaphylactic	2.6100	0.43841
	Anaphylactic	5.0000	0.14142
	Total	3.8050	1.40526
15 h	Non-anaphylactic	1.8300	0.01414
	Anaphylactic	3.2800	0.05657
	Total	2.5550	0.83728
18 h	Non-anaphylactic	1.3100	0.21213
	Anaphylactic	2.6400	0.00000
	Total	1.9750	0.77758
21 h	Non-anaphylactic	1.0800	0.16971
	Anaphylactic	1.9600	0.50912
	Total	1.5200	0.59509
24 h	Non-anaphylactic	0.8000	0.00000
	Anaphylactic	0.8700	0.35355
	Total	0.8350	0.20809

The quality of positive results in the non-anaphylactic and anaphylactic groups differed during the 15-hour post-mortem interval. In the non-anaphylactic and anaphylactic groups, there was a moderate accumulation of transudate fluid in the lumen of the alveoli and bronchioles. The bronchiolar integrity appears incomplete, with just a faint positive immunoreactivity. The non-anaphylactic group's mast cell granules were clearly visible intracytoplasmically.

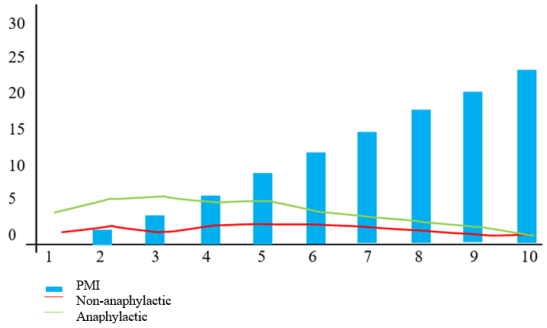

Figure 7. Descriptive curves of the lung mast cell chymase at different post-mortem intervals.

Table 3. Results of the univariate analysis of the lung mast cell chymase.

	Contrast	Error
Sum of squares	6.366	0.029
df	1	2
Mean square	6.366	0.051
F	433.590	
Sig.	0.0020	
Partial eta squared	0.995	

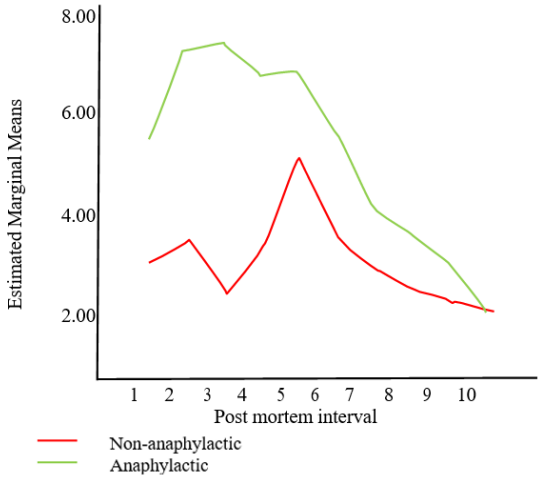

Figure 8. Estimated marginal means of mast cell tryptase.

A statistical analysis utilizing a multivariate repeated measure test revealed that there was an effect of the treatment on the mast cell chymase expression in the laryngeal organs, which was observed at different post-mortem intervals. Table 2 exhibits the mast cell chymase expression in the lungs of the experimental animals.

DISCUSSION

This study analyzed the expressions of lung mast cell β-tryptase and chymase due to anaphylactic shock, which were observed at different post-mortem intervals. This research employed rabbits (*Oryctolagus cuniculus*) that provided three types of specimens, i.e., the lungs, larynx, and heart.

A previous experimental laboratory research project employed a randomized block design (RBD) with equal subjects and a time series. The purpose of the research was to prove the relationship between the post-mortem interval and changes in immuno-histochemical features by analyzing theexpression of mast cell β-tryptase and chymase proteins in rabbit lung organs that experienced anaphylactic shock. Blood vessels of the rabbits (*Oryctolagus cuniculus*) were used as a scientific input to establish a precedent for mortality cases due to anaphylactic shock (Turner et al. 2017). The experimental animals had a one-week acclimatization period. Pellets were used for feeding, and drinking water was available ad libitum or without restriction. Clinical pathological examination of the complete blood picture of the rabbits was carried out to assess the presence of infection or other clinical indications that could interfere with the results of the study.

A study found a 25% prevalence of type I hypersensitivity among patients with drug-related hypersensitivity (Isyroqiyyah et al. 2021). Although the prevalence might appear to be low, this could lead to life-threatening consequences. Another study found that medications, such as antibiotics, were the most common cause of anaphylactic reactions (Hasanah et al. 2020).

A forensic expert is required in a variety of situations concerning the body, health, and life. The expert can identify corpses, human body parts, body conditions, time of death, cause of death, and provide a variety of written statements.

Strength and limitations

This study revealed that the expressions of tryptase and chymase at different post-mortem intervals could be utilized by forensic science technicians and experts as markers of anaphylactic shock. However, because this study was conducted in a controlled environment, the results may alter if other conditions are applied.

CONCLUSION

Forensic science technicians and experts can use tryptase and chymase expressions at different post-mortem intervals as anaphylactic shock (non-anaphylactoid) markers in the lungs, with varying mast cell chymase and tryptase production amounts and qualities.

Acknowledgment

The authors would like to thank Universitas Airlangga for providing the infrastructure that allowed this study to be conducted.

Conflict of interest

None.

Ethical consideration

This study was approved by the Research Ethics Committee of the Faculty of Veterinary, Universitas Airlangga, Surabaya, Indonesia, with the reference number 2.KE.157.08.2019 on 08/08/2019.

Funding disclosure

None.

Author contribution

BAP, IS, and AY contributed in gathering and analyzing the data. All authors contributed to the preparation and approval of the manuscript for publication.

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Case Report/Case Series

SURGICAL SITE INFECTION CAUSED BY NON-HEMOLYTIC *Staphylococcus aureus* FOLLOWING A TOTAL KNEE ARTHROPLASTY AT A CLASS C HOSPITAL IN INDONESIA

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ABSTRACT

This article presents a case report of a knee arthroplasty surgical site infection caused by non-hemolytic *Staphylococcus aureus*. A 56-year-old woman came to the Orthopedic Outpatient Clinic, with the chief complaint of pain in the left knee for the last three years. After being diagnosed with grade IV osteoarthritis, the patient underwent a total knee arthroplasty. The patient had routine post-operative follow-ups at the Orthopedic Outpatient Clinic. However, the patient complained of swelling, pain, and discharge at the surgical site after three months. The patient underwent a second surgery for debridement, implant removal, and interspacer placement. A broad-spectrum antibiotic (gentamicin) was administered while waiting for the culture and antibiotic sensitivity test results. The culture results showed non-hemolytic *Staphylococcus aureus* presence. The antibiotics were then changed and administered for two weeks according to the culture and antibiotic sensitivity test results. As the results were good, the patient was scheduled for revision surgery for her previous total knee arthroplasty.

Keywords: Surgical site infection; non-hemolytic *Staphylococcus aureus*; total knee arthroplasty; human and health

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Article history
●Submitted 16 Dec 2022 ● Revised 11 Jan 2023 ● Accepted 10 Feb 2023 ● Published 10 Mar 2023

How to cite: Satiyo & Rosa EM (2023). Surgical site infection caused by non-hemolytic *Staphylococcus aureus* following a total knee arthroplasty at a class C hospital in Indonesia. Folia Medica Indonesiana, 59 (1), 57-62. https://doi.org/10.20473/fmi.v59i1.39745

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. A patient with grade IV osteoarthritis underwent an arthroplasty and developed a surgical site infection caused by *Staphylococcus aureus.*
2. The treatment for the surgical site infection included antibiotic medications and revision surgery for the previous total knee arthroplasty.

INTRODUCTION

Surgical site infection (SSI) is a microbial infection at the surgical site that occurs within a period of less than 30 days up to one year after surgery (World Health Organization 2019). Surgical site infection is one of the most common types of nosocomial infections, which occur when a patient receives medical care in a hospital or other health care facility. Symptoms that can occur when a patient experiences a surgical site infection include purulent drainage, wound dehiscence, fever, pain or tenderness when pressure is applied, local swelling, and redness. Any of these symptoms can be found during physical examinations or supporting examinations, such as histopathological and radiological examinations (Wenk et al. 2017).

The surgical site infection prevalence among post-operative orthopedic patients is approximately 71%. The incidences of surgical site infection were 1.69% in orthopedic patients who underwent total hip replacement and 2.82% in those who underwent total knee replacement. The surgical site infection prevalence is generally low and closely related to the operating room layout, the surgical technique used by the operator, and the aseptic procedures carried out in a strict and disciplined manner (Ashraf et al. 2018, Saffanah et al. 2020). Total knee arthroplasty is a common surgery performed in the orthopedic

field to treat osteoarthritis, arthritis deformans, and rheumatoid arthritis. Recent studies found prevalent cases of knee osteoarthritis among people older than 55 years (Rositaet al. 2021, Gan et al. 2023). Total knee arthroplasty replaces the abnormal knee joint with an artificial material. The end of the femur is replaced with a metal shell, and the end of the tibia with a metal rod, both of which are then connected with plastic to act as a motion cushion.

A previous study found a 0.5%-2.0% probability of surgical site infection among patients undergoing total knee arthroplasty for the first time (Springer & Scuderi 2013). Surgical site infection will increase patient morbidity and mortality due to longer hospitalization, higher treatment costs, and a heavier mental burden on the patient (Purba et al. 2018, 2020). In order to treat surgical site infection, patients must undergo multiple procedures. Prior studies revealed that methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), and coagulase-negative *Staphylococci* are the most common causes of infection in total knee arthroplasty (Weiser & Moucha 2015).

CASE REPORT

A 56-year-old woman with the chief complaint of left knee pain that had persisted for the past three years presented to the Orthopedic Outpatient Clinic, Kertosono Regional Hospital, Nganjuk, Indonesia. The patient was diagnosed with grade IV osteoarthritis through clinical and radiological assessments. Figure 1 shows the x-ray of the patient's left knee.

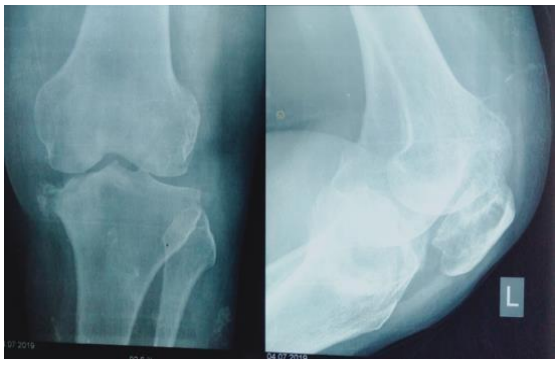

Figure 1. X-ray of the patient's left knee when first presented to the Orthopedic Outpatient Clinic.

After the diagnosis was confirmed, the patient underwent a total knee arthroplasty. Figure 2 presents the x-ray of the patient's left knee after surgery.

The patient presented for the post-operative follow-up a week after the surgery, and the evaluation results were good. However, three months following the surgery, the patient returned for follow-up with complaints of swelling, pain, and discharge at the surgical site. An x-ray was performed on the patient's left knee, as shown in Figure 3.

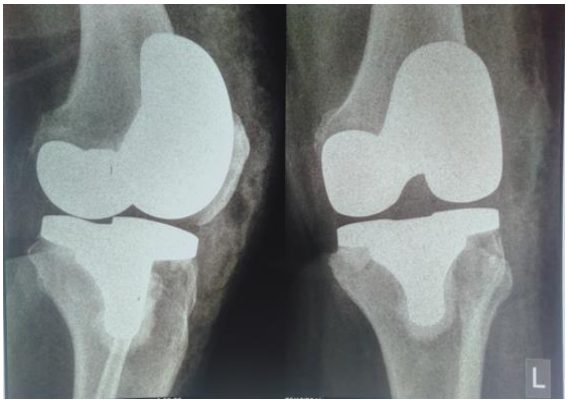

Figure 2. X-ray of the patient's left knee after total kneearthroplasty.

The patient was then advised to undergo a second procedure for debridement, removal of the previously inserted implant, and placement of an interspacer. Initially, the patient declined and requested outpatient treatment.

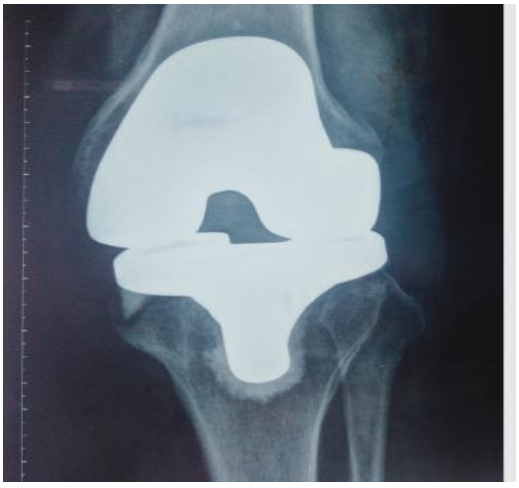

Figure 3. X-ray of the patient's left knee at three months after total knee arthroplasty.

After six months, the patient eventually agreed to undergo surgery. The surgery was therefore performed, as seen in Figure 4.

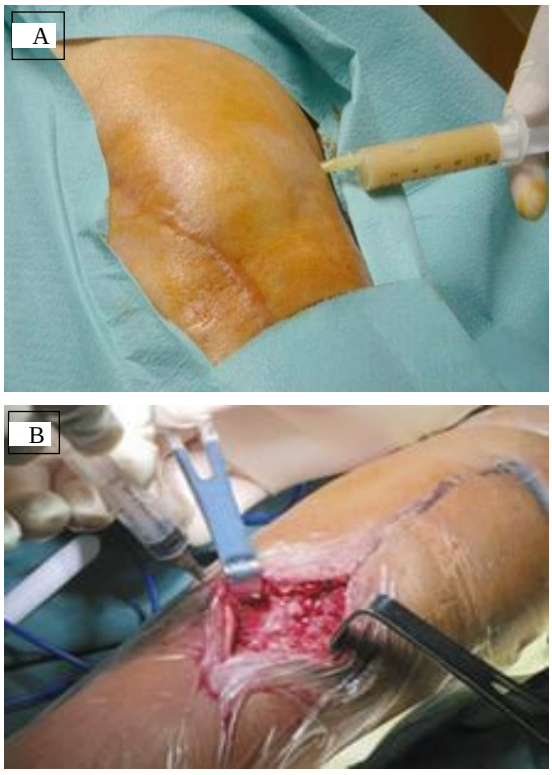

Figure 4. The patient's left knee before debridement and implant removal (A) and during debridement (B).

While waiting for the results of the culture and antibiotic sensitivity tests, the patient was administered a broad-spectrum antibiotic (gentamicin). Figure 5 depicts the x-ray of the patient's left knee after the second surgery.

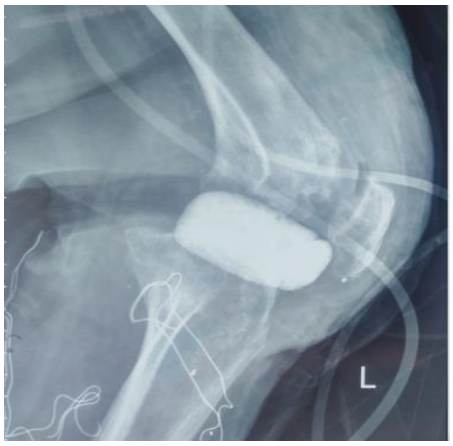

Figure 5. X-ray of the patient's left knee after debridement,implant removal, and interspacer placement.

A week following the second surgery, the results of the culture and antibiotic sensitivity tests came out, as shown in Table 1. A non-hemolitic Staphylococcus infection was discovered in the patient. The antibiotic medications were then adjusted according to the results of the culture and antibiotic sensitivity tests and administered for two weeks. The patient attended the post-operative follow-up a month following the second surgery. The evaluation results were satisfactory. She was then scheduled for a revision surgery for her prior total knee arthroplasty.

Table 1. Results of the culture and antibiotic sensitivity tests.

Material: Pus
Objective: AcrB culture = Antibiotic sensitivity testing
Culture: Biological organism: Non-hemolytic *Staphylococus*

Antibiotic sensitivity test:					
No.	Antibiotics		No.	Antibiotics	
I	Penicillin		V	Aminoglycoside	
	Ampicillin			Amikacin	R (0)
	Penicillin-G			Eictuamyca	
	Amoxycillin			Dibekacin	
	Oxacillin			Kanamycis	
II	β-lactamase inhibitor		VI	Tetracyclin	
	Ampicillin-Sulbactam			Tetracyclin	
	Amoxycillin-Clavulanic acid	S	VII	Phenicol	I (13)
	Ticancillin-Clavulanic acid			Chloramphenicol	
	Celoperarone-Salbactam		VIII	Macrolide	
	Piperacillin-Tazobactam	S		Erythromycin	
III	Cephalosporin			Clindamycin	R (0)
	1st generation		IX	Fluoroquinolone	
	Cepharolin			Ciprofloxacine	R (0)
	Cephalotin			Ofloxacin	
	Cephradine			Gatlonacis	
	2nd generation			Levofloxacin	
	Cefaroxime			Norfloxacin	
	Cefotiamin			Moxfloxacin	R (14)
	Cefoprozil	S	X	Carbapenem	
	3rd generation			Imipenem	S
	Cefoperaxone			Meropenem	
	Cefotaxime	S			
	Ceftriaxone		XI	Glycopeptides	
	Ceftazidime			Vancomycin	
	Cefpododime			Teicoplanin	
	Cefixine		XII	Others	
	4th generation			Fosfomycin	
	Celepime	S		Nalidoic acid	
	Cefpirom			Onezolid	S (33)
IV	Sulfa-Trimetoprime				
	Trimetoprime sulfametoksazole	R (0)			

S: sensitive; R: resistant

DISCUSSION

The patient in this study was a 56-year-old woman with no known comorbidity. Several studies on surgical site infection risk factors found that age and female sex are not risk factors for surgical site

infection. On the other hand, studies discovered that advanced age was a protective factor, but male sex was a risk factor for surgical site infection (Baier et al. 2019, Resende et al. 2021, Li et al. 2022). The patient in this study developed grade IV osteoarthritis, which necessitated a total knee arthroplasty. Because of its high success rate and low complication rate, total knee arthroplasty is the surgical procedure of choice for patients with end-stage knee arthritis. However, there were studies that reported 27% of surgical site infection cases within 30 days and 65% within a year after total knee arthroplasty (Lin et al. 2018, Chung et al. 2021).

The patient in this study experienced pain, swelling, and discharge at the surgical site. These clinical symptoms are considered in the diagnosis of surgical site infection. A study found that pain was the main symptom in >90% of cases (Zahar & Sarungi 2021). Following a clinical examination, supporting tests (such as laboratory and radiological examinations) are required to confirm the diagnosis. Plain radiography and blood tests are among the tests that must be performed. Two-plane radiography is utilized to check for solid implant fixation (radiolucent lines), osteolytic changes in the distal femur or proximal tibia, and periarticular ossification. During a blood test, the leukocyte count, differential, C-reactive protein (CRP) level, and erythrocyte sedimentation rate (ESR) are all checked (Cooper & Valle 2014, Shahi & Parvizi 2015). Despite these various tests, bacterial culture remains the gold standard for surgical site infection diagnosis (Goswami et al. 2018, Mühlhofer et al. 2021).

Previous studies indicated that methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), and coagulase-negative *Staphylococci* were the most common bacterial pathogens causing SSI after total knee arthroplasty. In surgical site infection cases, *Staphylococcus* accounted for 70% to 80% of the bacteria causing infection. Other bacteria commonly found in surgical site infection are Gram-negative bacilli and non-group A *Streptococcus* bacteria (Weiser & Moucha 2015, Gehrke et al. 2015). Several studies attempting to investigate the process of *S. aureus* colonization at the surgical site discovered that patients who had *S. aureus* nasal colonization were more at riskto develop a surgical site infection following total knee arthroplasty. The risk increased up to nine times compared to people who did not have nasal *S. aureus* colonization. Further molecular typing revealed that the *S. aureus* isolates from both sites (the surgical site and the nose) were molecularly identical (Skråmm et al. 2014).

Other than the case in this report, there was anothercase of surgical site infection at Kertosono Regional Hospital, Nganjuk, Indonesia. The patient had aprosthetic joint implanted and developed an infection caused by non-hemolytic *Staphylococcus*. Initial prosthetic joint infection occurs within three weeks after a procedure, whereas the occurence after those three weeks is considered late infection regardless of the stability of the components. A prosthetic joint infection indicates an infection not only at the prosthetic interface, but also in the surrounding bone and soft tissue (Zmistowski 2014). Early prosthetic joint infections are generally managed by aggressive debridement, exchange of modular parts, and retention of fixed components. Conversely, late prosthetic joint infections usually require the removal of the component (Osmon et al. 2013; Zmistowski 2014). The aforementioned patient at the Kertosono Regional Hospital had a late prosthetic joint infection. Several surgical options to treat PJI include debridement, antibiotics, and implant retention (DAIR), as well as single-stage and two-stage revision surgery (Karachalios et al. 2014, Karachalios & Komnos 2021). If all of the patient's reconstructive treatments fail, salvage surgeries (e.g., arthroplasty resection, fusion, and above-the-knee amputation) can be undertaken (Gehrke et al. 2015).

In addition to surgical care, patients must be administered antibiotics to treat the infection. Antibiotics were administered post-operatively in somecases to prevent surgical site infection (Hadi & Ishardyanto 2021). In general, antimicrobial therapy should be pathogen-directed and guided by the results of antimicrobial susceptibility testing where applicable (Osmon et al. 2013, Tande & Patel 2014). The general principles for antimicrobial treatment are to apply the least toxic, most efficacious, narrow-spectrum antimicrobial regimen preferentially. When several agents are deemed equal, the cost and convenience of the administration should also be considered (Chaussade et al. 2017, Bernard et al. 2021).

Strength and limitations

This case report can contribute to recent studies on surgical site infection by identifying the bacteria causing the infection that occurred to the patient in this study. The findings of this study confirm that *Staphylococcus aureus* is among the bacteria most commonly causing surgical site infections. This study also further confirms that treatments for surgical site infection require a second procedure for debridement, implant removal, and interspacer placement. However, this case study may not be able to generalize and demonstrate a cause-effect correlation of the infection.

CONCLUSION

Staphylococcus aureus was found to cause surgical site infection investigated in this study. Treatments for surgical site infection as a post-operative complication are important and should be carried out immediately, which may include debridement, antibiotic medications, and revision surgery.

Acknowledgment

The authors would like to thank the Department of Orthopedics, Kertosono Regional Hospital, Nganjuk, Indonesia and the Postgraduate Program of Hospital Administration, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia.

Conflict of interest

None.

Funding disclosure

None.

Author contribution

STY and EMR participated equally in the design of this study, manuscript preparation and drafting; proposed the main idea; as well as contributed equally to the study design, methodology, supervision, and formal analysis.

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Review Article

THE HISTOPATHOLOGICAL FEATURES OF SYPHILIS AND ITS MIMICKERS

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ABSTRACT

Syphilis, also known as "the great imitator," is a sexually transmitted infection with a variety of clinical symptoms and histopathological similarities to other infectious diseases. Public health concerns about syphilis have grown significantly. Since 2000, there has been an increase in syphilis prevalence in the United States, with a 17.6% increase from 2015 to 2016. From 2000 to 2019, the number of syphilis cases throughout Asia increased from 0.9% to 30.9%, whilst the number of cases in Indonesia decreased from 22.5% to 14.4%. Specific serological tests for syphilis can usually detect and confirm the diagnosis and offer follow-up care in most cases. However, in certain instances, the clinical characteristics discovered during testing can be identical to those of other diseases, which may lead to inconsistent diagnosis. Considering that the diagnostic pathology is pertinent to the clinical circumstances, a histopathological investigation may be useful for differentiating syphilis mimickers. Pathology is essential for identifying potential syphilis patients with ambiguous clinical symptoms. This study's purpose was to assist dermatologists and pathologists in identifying "mimickers" that require a biopsy and in determining the correct diagnosis and treatment coutsed based on etiology.

Keywords: Syphilis; mimickers; dermatopathology; infectious disease; sexually transmitted infection

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Article history
●Submitted 10 Dec 2022 ● Revised 12 Jan 2023 ● Accepted 22 Feb 2023 ● Published 10 Mar 2023

How to cite: Purnamasari I, Hidayati AN, Kusumastuti EH, et al (2023). The histopathological features of syphilis and its mimickers. Folia Medica Indonesiana, 59 (1), 63-69. https://doi.org/10.20473/fmi.v59i1.37286

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. The importance of having a strong suspicion for syphilis and maintaining close contact between dermatologists and pathologists cannot be understated.
2. Understanding the clinical relationship and histopathological features of syphilis is crucial for accurate diagnosis and distinction from its histopathologic mimickers.

INTRODUCTION

Syphilis is a chronic and systemic spirochete bacterial infection brought on by the *Treponema pallidum* subspecies *pallidum*. Syphilis spreads through microabrasions in the mucosa or skin, which occur almost exclusively during sexual contacts. It can spread rapidly through the bloodstream to the tissues. Additionally, syphilis can be transmitted through blood transfusions and from mother to child through the placenta (Ho & Lukehart 2011, Ali et al. 2016, Wardiana et al. 2022).

Syphilis is a global public health problem, especially in high- and middle-income countries. The World Health Organization (WHO) reported global estimates of 12 million new syphilis cases each year and one million pregnancies complicated by the disease. In recent years, the syphilis incidence has still been high, especially in Africa, Asia, and Central and South America (World Health Organization 2017, Tuddenham & Zenilman 2019).

Data from the Centers for Disease Control and

Prevention (2017) showed that there was a 52% increase of cases among men who have sex with men (MSM), with 30-74% of them co-infected with human immunodeficiency virus (HIV). Whereas, the of syphilis prevalence in Indonesia among MSM groups showed an increase from 8.5% in 2011 to 15.7% in 2015 (Daili et al. 2015).

Syphilis is called "the great imitator" because it has widely varying clinical presentations and histopathological features that also exist in other infectious diseases (Tuddenham & Zenilman 2019). Lesions in early syphilis can be mistaken as those of other infections and conditions; therefore, syphilis should be suspected in all sexually active patients presenting with a new skin rash or an oral/genital lesion (Klausner 2019). Without treatment, syphilis can be associated with significant morbidity and mortality, especially when transmitted vertically from mother to child or in patients with advanced tertiary disease. If syphilis is left untreated, the infection may proceed through a multistage process of primary, secondary, and tertiary stages. However, it is known that an initial syphilis infection can also heal spontaneously (Klausner 2019, Tuddenham & Zenilman 2019).

Proper and reliable examination is essential for establishing the correct diagnosis of syphilis and the accuracy of the medication administration, especially in latent syphilis. Universal screening and adequate pregnancy care are important for preventing syphilis in mothers and its transmission to their children (Purnamasari et al. 2021). In addition to careful review of sexual history and physical examination, several methods can be useful in establishing the clinical diagnosis of syphilis. Today, in most cases, it can be diagnosed and followed up due to specific serological tests (RPR, VDRL, *T. pallidum*-antibody), and recently with PCR as well. In cases with atypical clinical presentations and false-negative serological laboratory results, a biopsy examination can assist in establishing clinically relevant diagnosis (Hook 2017, Tuddenham & Zenilman 2019).

OVERVIEW

The incidence of primary and secondary syphilis in the United States is at its highest level since 1994, negating the dramatic decline that was seen when HIV infection first emerged and many people's sexual habits changed as a result (Kojima & Klausner 2018). In Indonesia, syphilis is still prevalent at a high rate. Syphilis still affects 25% of transgender people. Its frequency has increased three times among MSM and injectable drug users since 2007. The prevalence of syphilis is 25% in women who sell direct sex (female sex workers/FSW), 10% in MSM, 9% in prisoners, 5% in high-risk men, and 3% in female indirect sex workers (Daili et al. 2013, Gunn & Klausner 2019). People who were infected with HIV and syphilis could improve their quality of life by actively participating in programs, such as anti-retroviral therapy and counseling, although reducing the prevalence of the disease should be of the utmost importance (Yuindartanto et al. 2022).

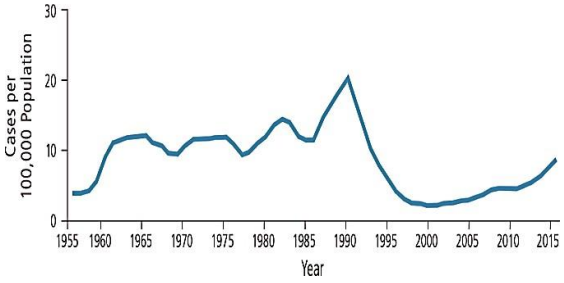

Figure 1. New cases of primary and secondary syphilis in the United States, 1956 to 2016 (Kojima & Klausner 2018).

Although syphilis can be identified based on its clinical stage, it has the ability to mimic a variety of other diseases at any stage of its development (Hook 2017). Figure 2 shows the natural stage of syphilis.

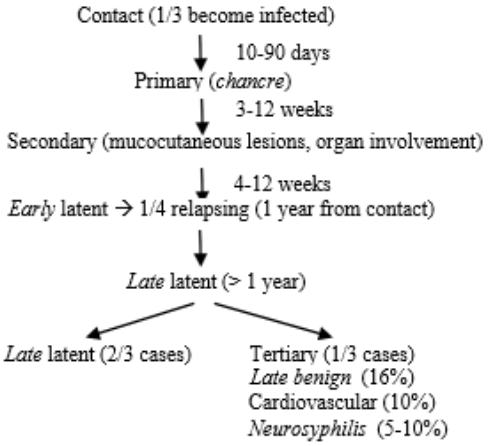

Figure 2. The progressions of syphilis related to its stages (Tuddenham & Zenilman 2019).

The role of biopsy in distinguishing syphilis mimickers

The diagnosis of syphilis should include the consideration of the clinical, serological, and histopathological findings (Groh & Patino 2014). The statistical data from the Centers for Disease Control and Prevention (CDC) shows that the total rate of syphilis diagnosed with biopsy increased significantly since 2003 (Figure 3). However, in

most dermatopathology laboratories, only about 32% of the syphilis diagnoses were resulted from biopsy. Biopsy examination is one of the helpful and accurate tools used in diagnosing syphilis, evaluating the course of syphilis, and confirming the correlation of the clinical morphology, serology, and histopathology (Flamm et al. 2015). Histologically, typical presentation of syphilis depends on the stage and type of lesion seen from the biopsy. Although it is not the gold standard for diagnosis, the presence of *T. pallidum* spirochetes in the histopathology can indicate syphilis. Ghaznawie (2013) stated in a study that silver staining or immunohistochemical staining can detect spirochetes. Two fundamental pathological changes in syphilis are proliferation and swelling of endothelial cells, and a perivascular infiltration of lymphoid cells and plasma cells. Treponemes are commonly seen in both primary and secondary lesions (Elder 2017).

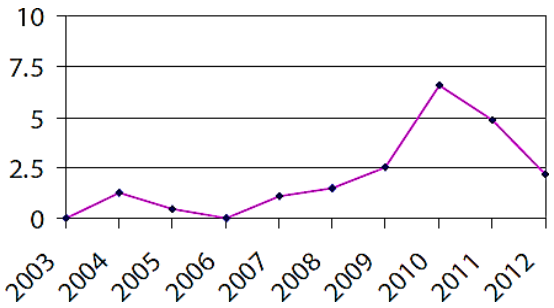

Figure 3. The total rate of biopsy diagnosis of syphilis according to the CDC data (rates per 100,000 biopsies) (Groh& Patino 2014).

Histopathological features in syphilis "the mimicker"

The classic manifestation of primary syphilis is a painless and well-circumscribed ulcer with an indurated base and raised borders, which known as "durum ulcer" (Tuddenham & Zenilman 2019). However, atypical symptoms or morphology may present, causing diagnosis challenging. Some techniques used in syphilis diagnosis are dark-field microscopy and Warthin-Starry staining. Spirochetes are found along the dermal-epidermal junction, inside and around the blood vessels, using immunofluorescent techniques and silver staining with the Levaditi stain or Warthin-Starry stain (Figure 4b). Endothelial swelling (endarteritis obliterans), ulceration, and a diffuse dermal infiltrate of plasma cells, lymphocytes, and histiocytes are the main histopathological findings at this stage (Figure 5a) (Johnston 2012, Elder 2014).

Many clinical symptoms of first stage syphilis have similar features as other diseases, such as chancroid or mole ulcer, genital herpes, lymphogranuloma venereum, and granuloma inguinale (Çakmak et al. 2019). Chancroid is the most similar mimicker that is difficult to distinguish from syphilis chancre. Chancroid is an acute localized genital infection caused by *Haemophilus ducreyi*, with necrotic ulcer, pain at the site of inoculation, and tender ulcer as the classic clinical symptoms (Figure 5A) (Irizarry et al. 2021). The histopathological characteristics of chancroid are solid lymphohistiocytic infiltrates with a lack of plasma cells and granulomatous vasculitis. There are three zones at the base of chancroid ulcer, i.e., an upper layer with tissue necrosis, fibrin, and neutrophils; a middle layer with vascular proliferation, prominent endothelial cells, and mixed infiltrate cells; and a deep zone with plasma cells and lymphocytes (Figure 5B, Figure 5C) (Johnston 2012).

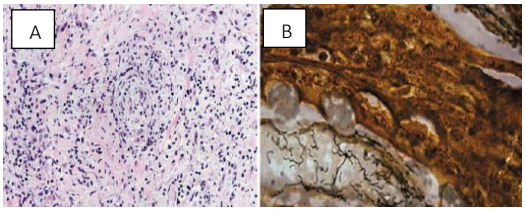

Figure 4. Primary syphilitic chancre, (a) endarteritis obliterans and diffuse dermal infiltrate of plasma cells, lymphocytes, and histiocytes; (b) the observation of *Treponema* morphology using silver stains (Johnston 2012).

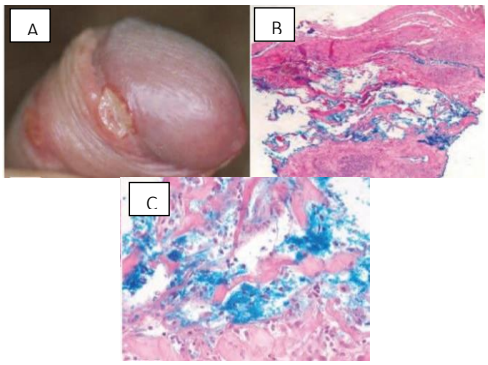

Figure 5. Chancroid ulcer with well-defined margins in the penile coronal sulcus (A); spongiform pustulation, psoriasiform changes, extensive ulceration, and three zones at the ulcer base (B, C) (Johnston 2012, Tuddenham & Zenilman 2019).

Herpes simplex virus (HSV)-induced genital herpes is an infection of the genitalia, with recurrent vesicles that cluster on an erythematous base as the characteristic symptoms. These vesicles are prone to rupture that can result in numerous erosions, ulcerations, and alterations, such as diffuse inflammation and massive necrotic ulceration

(Figure 6A) (Holmes et al. 2008). The simplest laboratory examination to distinguish HSV from a chancre is the Tzanck test with Giemsa or Wright stains, which will reveal the multinucleated giant cells. However, this test generally has a low sensitivity and specificity. The best examination of HSV is by tissue culture and enzyme linked immunosorbent assay (ELISA) (Holmes et al. 2008, Zhu & Viejo-Borbolla 2021). The histopathological features of genital herpes include ballooning degeneration of keratinocytes, multinucleated giant cells with nuclear folding, basophilic eggshell in the periphery of the nucleus as the cytopathic effect of herpes, and mild leucoclastic vasculitis (Figure 6B, Figure 6C) (Ghaznawie 2013, Elston et al. 2014, Elder 2017).

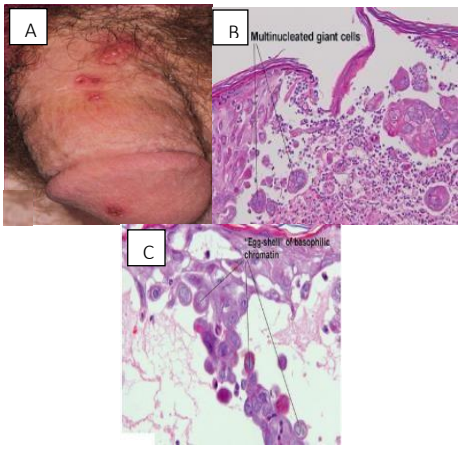

Figure 6. Clinical manifestation of genital herpes shows numerous vesicles that cluster on an erythematous base, with multiple ulcerations and necrotic ulcers (A); ballooning degeneration of keratinocytes and multinucleated giant cells (B); basophilic eggshell of chromatin at the periphery of the nucleus (C) (Elston et al. 2014, Tuddenham & Zenilman 2019).

Lymphogranuloma venereum (LGV) is a systemic sexually transmitted disease caused by *Chlamydia trachomatis* serovars L1, L2, and L3. The clinical manifestations of LGV are divided into three stages, the primary, secondary, and tertiary stages. Lesions will develop 3–30 days after infection, in the form of small herpetiform ulcers at the inoculation site and painful ulcers. Nonspecific ulceration and urethritis may occur rarely (Figure 7A). After a few weeks, primary-stage LGV will transition into a secondary stage, which is characterized by signs of lymph vessel involvement and hematogenous spread (Figure 7B) (Holmes et al. 2008). Histopathological features of the LGV mimicker show the non-specific granulation tissue. In the lymph nodes, a stellate abscess with surrounding epithelioid cells and macrophage giant cells represents the typical lesion (Figure 7C) (Johnston 2012). The prevalence of *Chlamydia trachomatis* as the most causal organism of non-specific genital infection is rare. However, the examination of the cause of non-specific genital infection is still required to register the exact treatment and not to confuse the disease as other conditions (Habibie et al. 2019).

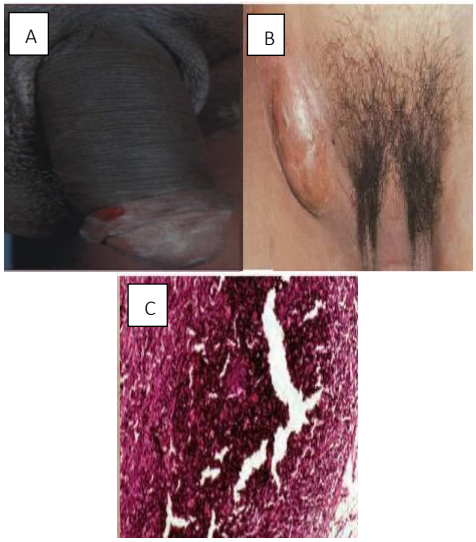

Figure 7. Clinical primary stage with small herpetiform ulceration (A); secondary stage with lymph involvement, hematogenous spread (B); a stellate abscess with surrounding epithelioid cells and macrophage giant cells (C) (Johnston 2012).

Granuloma inguinale (donovanosis) is a sexually transmitted infection caused by *Klebsiella granulomatis* (*Calymatobacterium granulomatis*). The disease is characterized by beefy red lesion in the form of ulcer filled with abundant granulation tissue, that bleeds easily. The boundaries of the ulcer are clear and have serpiginous lines (Figure 8A). The histopathological figures of a granuloma inguinale mimicker show pseudopitheliomatous hyperplasia with neutrophilic abscess (Figure 8B) and organisms in histiocytes of the dermis (Donovan bodies) (Figure 8C). Electron microscopy showed that the organisms were very thin with big green leafy veggie appearance (Johnston 2012, Elder 2017).

Secondary syphilis is known clinically as the "great imitator" because it resembles a variety of skin conditions and manifest itself anywhere on the body, including the palms of the hands and soles of the feet. Significant symptoms that are important to note in distinguishing syphilis from other diseases are skin conditions in the secondary stage, which are generally not itchy, often accompanied by generalized lymphadenitis, and also occur on the palms and soles (Johnston 2012, Elder 2017, Tuddenham & Zenilman 2019). Histopathological features of secondary syphilis lesions (such as macular, papular, and papulosquamous types) often overlap. However,

epidermal changes are rare in the clinical form of macular and papulosquamous lesions (Elder 2017).

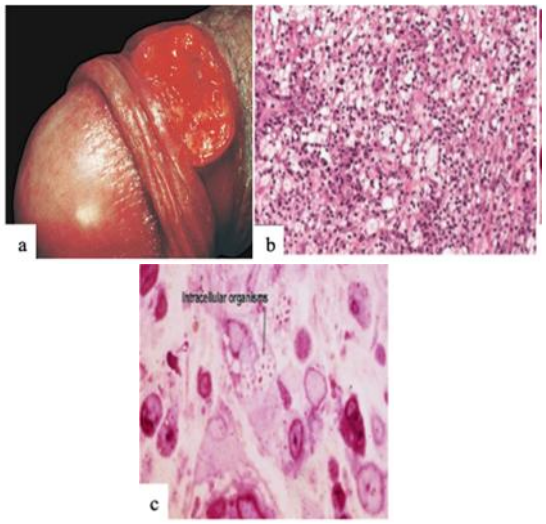

Figure 8. Neutrophilic abscess cells mixed with foamy macrophages (A); Donovan bodies with pseudoepitheliomatous hyperplasia; (B) clinical manifestation of ulcer characterized by a beefy appearance (C) (Elston et al. 2014).

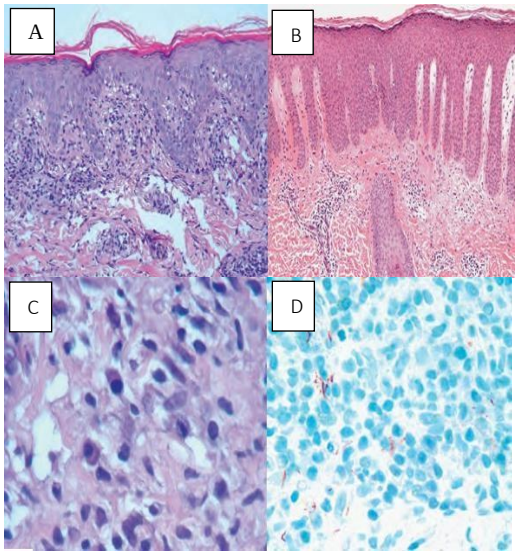

Figure 9. Histopathological features of psoriasis: neutrophils in the epidermis, regular acanthosis, prominent papillary blood vessels (x5) (A); pityriasis rubra pilaris: checkerboard-pattern hyperkeratosis, parakeratosis, and follicular blockage (x10) (B); secondary syphilis: acanthosis with slender rete, lichenoid inflammation, endothelial cell swelling, plasma cells occasionally seen (x5) (C); chronic spongiotic dermatitis: parakeratosis and spongiosis, irregular acanthosis (x5) (D) (Ko & Barr 2017).

The most common histopathological sign of secondary syphilis is psoriasiform hyperplasia, which is frequently accompanied by spongiosis and vacuolar alterations. Other features may vary, including the presence of parakeratosis, abundant plasma cells, edema of the papillary dermis, lichenoid, and granulomatous. *Treponema pallidum* can be identified by silver staining, such as the Warthin-Starry stain or immunoperoxidase technique (Elston et al. 2014, Elder 2017). Various mimickers in secondary syphilis lesions that have psoriasiform histopathological features are difficult or impossible to distinguish, including psoriasis, pityriasis rubra pilaris, and chronic dermatitis (Johnston 2012, Elston et al. 2014, Ko & Barr 2017).

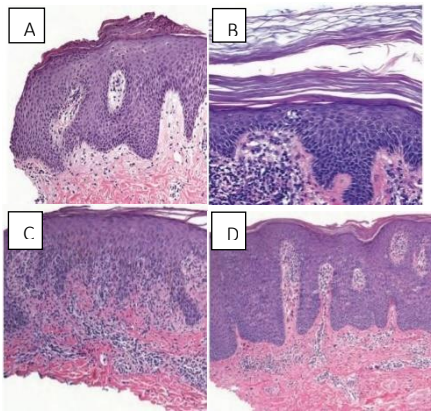

Figure 10. Psoriasiform hyperplasia seen mainly in the epidermis; orthohyperkeratosis, parakeratosis, and papillary dermal edema (x5) (A, B); vacuolar alterations with an abundance of neutrophils and barely perceptible plasma cells are observed (C); *T. pallidum* is visible in a silver-staining with 100x oil immersion (D) (Johnston 2012, Ko & Barr2017).

Strength and limitations

This review article discusses the histopathological characteristics that help distinguish syphilis from its common clinical and histological mimickers at each stage. However, because each research article analyzed in this study presented diverse cases with various symptoms, it was tricky to synthesize the distinguishing criteria. Therefore, universal screening and adequate pregnancy care must be a priority. It is recommended that future studies be discussed in greater detail with regard to the description of a number of additional syphilis mimickers, so that case recognition is better understood to aid in the diagnosis of syphilis.

CONCLUSION

High suspicion of syphilis and close communication between dermatologists and pathologists remain of the utmost importance. Dermatologists and

pathologists should take the variety of clinical and histologic presentations into account when making a differential diagnosis. The independent value of numerous syphilis histologic characteristics may have been overstated. Endothelial swelling (endarteritis obliterans), interstitial inflammation, irregular acanthosis, and elongated rete ridge combinations raise the probability of syphilis. Vacuolar interface dermatitis and lymphocytes with visible cytoplasm also raise syphilis probability. Understanding of syphilis's clinical correlation and the histological appearance is crucial to correctly diagnosing the disease and distinguishing it from the histologic mimickers.

Acknowledgment

The authors would like to thank the Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

Conflict of interest

None.

Funding disclosure

None.

Author contribution

IP, ANH, and EHK contributed to the conception of the study, and drafted the manuscript. IE gave final approval for the manuscript to be published, and agreed to be accountable for all aspects of the work.

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Systematic Review

THE IMPACT OF SOCIAL STIGMA ON CHILD PATIENTS WITH CLEFT LIP AND PALATE

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ABSTRACT

Social rejection still happens to a large number of children who have cleft lip and palate. Stigma has a detrimental impact on children with cleft in the aspects of education, employment, marriage, and community acceptance, which can be exacerbated by barriers to high-quality child care. A literature study related to this topic was conducted by searching for articles from 2018 to October 2021 through three databases (i.e., PubMed, Embase, and Medline). The term used for the literature search was "cleft", which generated results that were sorted alphabetically and by relevance. This yielded 15 articles that focused on social stigma's influence on cleft lip and palate patients. In conclusion, the articles generally reported that the awareness of social stigma impacts on children with cleft lip and palate are lacking in various areas. It is important to identify social stigma's influence on children with cleft lip. This can help surgeons prioritize resource allocation and provide further evidence in incorporating quality of life measures into the treatment outcome assessment.

Keywords: Social stigma; cleft lip; children; social determinant of health

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Article history
●Submitted 30 Nov 2022 ● Revised 10 Jan 2023 ● Accepted 2 Feb 2023 ● Published 10 Mar 2023

How to cite: Ariani Y & Diska A (2023). The impact of social stigma on child patients with cleft lip and palate. Folia Medica Indonesiana, 59 (1), 70-74. https://doi.org/10.20473/fmi.v59i1.39641

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. Awareness of social stigma impact on children with cleft lip and palate is lacking in various areas.
2. Identifying social stigma's influence on children with cleft lip is important to help surgeons prioritize resource allocation and provide further evidence in incorporating quality of life.

INTRODUCTION

One of every 700 children is born with a cleft lip, making it the most common congenital craniofacial anomaly in the world (Tanaka et al. 2012, Fell et al. 2014, Allori et al. 2017). The cleft lip and palate prevalence rates in children vary widely across socioeconomic class, gender, and ehtnicity. However, children with cleft lip and palate may encounter additional challenges in low- and middle-income countries (LMICs). Significant dis-advantages, such as speech difficulties, delays in physical and cognitive development, persecution by bullies, and social exclusion, can arise from cleft lip later in life.

Children who have cleft lip and palates are more susceptible to psychosocial influences, such as those from the community on their ability to learn, work, and even get married. If this persists into adulthood, higher-level work and education are more difficult to obtain for them (Camille et al. 2014, Mzezewa et al. 2014, Maine et al. 2017). There is currently no literature or scientific writing that analyzes the impact of social stigma on child patients in a variety of areas. Clarifying and identifying the effects of social stigma can help with resource allocation and gathering evidence to incorporate quality of life measures into outcome assessment in the care of children with cleft lip (Allori et al. 2017).

MATERIALS AND METHODS

This study followed the screening process guideline of systematic reviews and was registered in the

International Prospective Register of Systematic Reviews (PROSPERO). Three databases (i.e., PubMed, Embase, and Medline) were utilized in the literature search, limited to studies from 2018 to October 2021. The term "cleft" was used as a keyword in the literature search to produce maximum results, which were then sorted alphabetically and by relevance. Table 1 exhibits the literature search process using the Medical Subject Headings (MeSH) in order to yield article titles from the three databases. The inclusion criteria in this study were limited to literature discussing social stigma and cleft lip and palate in pediatric patients, who were no older than 18 according to the United Nations.

The literature search generated 477 entries, 32 of which included full-text articles. After reviewing the literature, 15 articles were identified to match the inclusion criteria of this study. Evidence-based theories were utilized to provide descriptive analyses, which were employed in the article review process. The selected literature reported several factors that would arise if cleft lip patients did not receive special care.

RESULTS

Only fifteen studies found in the literature search results that addressed the impact of social stigma on pediatric patients with cleft lip and palate (Table 2). These studies indicated a 47% involvement of international cooperation in the care of child patients with cleft lip and palate. The majority of the studies were conducted in India (n=4) and Nigeria (n=4). Several factors were investigated in the studies, i.e., public trust (60%), social influence (46%), marriage (46%), education (40%), employment (33%), and psychological distress (20%).

There is a belief in the society that cleft lip and palate are caused by the parents' actions or faults (Adeyemo et al. 2016), such as punishing their children with abuse to their faces (Maine et al. 2017). Children with cleft lip were frequently perceived as though they were not human (Conway et al. 2015). Most people realize that cleft lip affects only the lip and face. However, they do not realize its etiology and how to prevent it (Wong Riff et al. 2017).

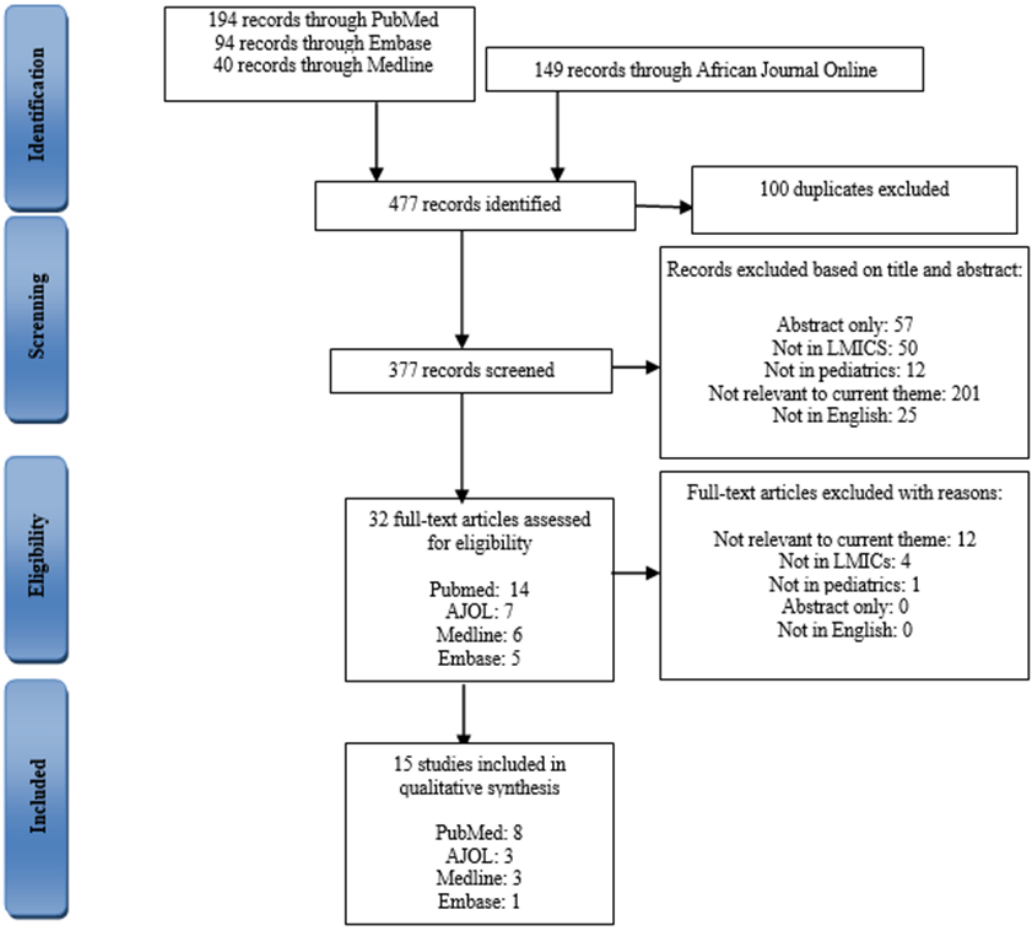

Figure 1. Screening process diagram of the literature search results.

Table 1. The Medical Subject Headings (MeSH) for the literature search through PubMed, Embase, and Medline.

	Where	Who	What
PubMed	Poverty Poverty area Developing country Social welfare Rural health Rural population Hospitals, rural Rural health service International agency Socioeconomic factor	Child under 18 years old	Cleft lip palate Cleft lip Cleft face Cleft lip Unilateral cleft lip Cleft lip face palate Cleft lip palate
Embase	Socio International cooperation Social welfare Developing country Poverty Rural health care Rural population Rural area Urban rural difference	Infant <to one year> Child <unspecified age> 1 to 6 years old child 7 to 12 years old child 13 to 17 years old child	Cleft lip Cleft lip palate Cleft face Cleft lip nose Unilateral cleft lip Cleft lip face palate Cleft palate
Medline	Poverty Poverty Develop country Social welfare Rural health Rural population Hospital Rural health service International agency Socioeconomic factor	Child aged 23 months Child aged 0 to 18 years Child aged 1 month Child aged 1 to 23 months Child aged 2 to 5 years Child aged 6 to 12 years Child aged 13 to 18 years	Cleft lip Cleft palate

Children with cleft lip and palate typically faced social repercussions, such as social isolation or being isolated from their surroundings (Yao et al. 2016, Wong Riff et al. 2017, Crerand et al. 2017, American Academy of Pediatric Dentistry 2019). Children with cleft lip and palate might be neglected by one or both parents since their physical appearance was seen as "terrifying" by their parents (Crerand et al. 2017, Hlongwa & Rispel 2018). However, several studies reported that there were parents who had a favorable attitude and interacted closely with their children who had cleft lip and palate (Worth et al. 2017, Soeselo et al. 2019).

Table 2. The selected literature on children with cleft lip and the influence of society.

Research	Type of study
Tanaka	Retrospective
Allori	Cross-sectional
Camille	Retrospective
Abid	Qualitative
El-Shazly	Qualitative
Fadeyibi	Qualitative
Fell	Qualitative
Klassen	Case study
Mzezewa	Cohort
Naram	Qualitative
Butali	Qualitative
Owotade	Cross-sectional
Parmar	Narrative
Bluher	Qualitative
Mednick	Qualitative

Many studies have examined how children with cleft lip and palate struggled to attend school. Children with cleft lip were not allowed to attend school due to their "terrifying" appearance, which was often not tolerated in school. They were either denied admission to school because their appearance would frighten other children or they refused to attend school due to bullying. Surgery might have benefits for children with cleft lip and palate to avoid these situations (Kumar et al. 2014, Ness et al. 2015, American Cleft Palate-Craniofacial Association 2017). Despite the original size and completeness of the cleft, surgery might help to establish the symmetry of nasal width, philtral height, horizontal lip length, and vertical lip height in cleft lip and palate patients (Datusanantyo et al. 2021).

In the studies reviewed, children with cleft lip and palate might endure psychological distress because they were perceived as a burden on their families. Furthermore, they regarded their parents as having negative feelings toward them, resulting in a higher proportion of children and adolescents with cleft lip and palate feeling depressed. Social dysfunction, which restricted their opportunity to interact with peers, also contributed to their psychological distress. The children were often not allowed to interact with other people at all, indicating a higher incidence of social dysfunction (Worth et al. 2017, World Health Organization 2018).

DISCUSSION

The social repercussions on children with cleft lip and palate stemmed from misleading cultural belief that those children were born with supernatural curse and would bring negative influence to people (Wong

Riff et al. 2017). This would result in bullying and, in some cases, family seclusion for those children. This also occurred in school causing children to drop out and encounter barriers to higher education. In adulthood, they would be seen as a burden as they struggled to be hired for paid employment.

Cleft lip and palate surgery and reconstruction could provide these children hope for education, employment opportunities, social acceptance, and improved marriage prospects. It is advised to seek surgical treatment as early as possible. However, a study found that a patient's age at the time of cleft palate surgery was strongly related to the parents' income and access to information (Ningrum et al. 2021). Adults with severely neglected cleft lip and palate could still be treated, but communication difficulties accompanying these conditions might persist (Arista & Hutagalung 2021). Providing education, support, and outreach to people with cleft lip and palate could help them to integrate into the societal system and change the social stigma (Conway et al. 2015, Crerand et al. 2017). Primary care, including treatments for children with cleft lip and palate, should include physical, psychological, and social components (Roosihermiatie et al. 2018).

Strength and limitations

The literature selected for this study had some limitations. Many of the articles did not employ the Patient-Reported Outcome Measures (PROMs), which might include questions about education, social impact, and psychological functioning for cleft lip patients. It is recommended to use the PROMs that can be a valid tool to assess and understand the impact of cleft lip cases and treatments. However, treatments for children with cleft lip and palate would only succeed if there is a solid cooperation between governments and non-governmental organizations to reduce social stigma and improve public education.

This study also had limitations because many cases of cleft lip and palate were unreported in some places. This review study covered only literature in English, so research published in other languages was excluded. To uncover further evidence of the social impact on children with cleft lip and palate, open discussion with colleagues from various regions is required.

CONCLUSION

Children with cleft lip and palate encounter social stigma, which has a major impact on their psychology and development. However, awareness of this phenomenon is still very lacking in many regions. The Patient-Reported Outcome Measures (PROMs) are recommended for assessing surgical treatment or reconstruction's effectiveness in reducing the psychosocial burden on children with cleft lip and palate, particularly in low- and middle-income countries (LMICs). Collaboration between governments and non-governmental organizations is also suggested to decrease social stigma and enhance public education as part of these children's healthcare.

Acknowledgment

None.

Conflict of interest

None.

Funding disclosure

None.

Author contribution

YA designed the study, gathered the data, and approved the final manuscript. DA designed the study and prepared the manuscript.

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Meta-Analysis

MORTALITY AMONG HEART FAILURE PATIENTS IN THE PRESENCE OF CACHEXIA

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ABSTRACT

Despite the fact that obesity has long been recognized as a risk factor for cardiovascular disease, the mortality rate of heart failure (HF) patients with cachexia is still high. Several studies have been conducted to investigate the association between cachexia and mortality in HF patients. However, the research results vary, as do the diagnostic criteria employed to assess cachexia. This meta-analysis aimed to conclusively summarize the association between cachexia and mortality in HF patients. The data were obtained from prospective or retrospective cohort studies with full texts in English or Indonesian and keywords related to "cachexia," "heart failure," and/ or "mortality". Studies that did not assess mortality in HF patients with cachexia and had no full text accessible were omitted. A literature search was conducted through four databases (PubMed, Web of Science, Scopus, and SAGE Journals) using keywords, reference searches, and/ or other methods on April 2022 in accordance with the Preferred Reported Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Data from the selected studies were presented and analyzed using qualitative and quantitative synthesis methods. The Newcastle-Ottawa Scale (NOS) was used to assess the risk of bias in the selected cohort studies. The qualitative synthesis contained nine studies, whereas the quantitative synthesis (meta-analysis) included six studies. Cachexia was found in 16.0% of the 4,697 patients studied. During the 180-1,876-day follow-up period, 33.0% of the patients died, with a mortality rate of 38.8% among the patients with cachexia. The pooled analysis revealed cachexia to be a significant predictor of mortality in HF patients (hazard ratio (HR)=3.84; 95% CI=2.28-6.45; p<0.00001), but with significant heterogeneity (p<0.00001; I^2=88%). In conclusion, cachexia worsens HF prognosis.

Keywords: Cachexia; heart failure; cardiovascular disease; mortality; well-being

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Article history
●Submitted 12 Nov 2022 ● Revised 15 Jan 2023 ● Accepted 21 Feb 2023 ● Published 10 Mar 2023

How to cite: Andrianto, Karman UNP, Mudjanarko SW, et al (2023). Mortality among heart failure patients in the presence of cachexia. Folia Medica Indonesiana, 59 (1), 75-84. https://doi.org/10.20473/fmi.v59i1.39512

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. Around 38.8% of heart failure patients with cachexia died during the 180-1,876-day follow-up period.
2. Cachexia increases the risk of mortality in heart failure patients.

INTRODUCTION

Heart failure (HF) is a complex clinical syndrome caused by structural or functional problems with ventricular filling or blood ejection (Ponikowski et al. 2016). HF patients may experience a variety of symptoms, including breathlessness, decreased exercise tolerance, and ankle swelling. This may be accompanied by signs such as elevated jugular venous pressure, gallop rhythm, pulmonary crackles, and peripheral edema (Yancy et al. 2013, Ponikowski et al. 2016, Bangsa et al. 2021). HF frequency varies greatly across the world, but its mortality rate remains around 50% following five years of diagnosis (Yancy et al. 2013, Virani et al. 2021).

Obesity and overweight are defined as abnormal fat accumulations that can be risk factors for the development of a variety of diseases. Obesity has long been recognized as a risk factor for any cardiovascular-related diseases (Yancy et al. 2013, Ponikowski et al. 2016). However, many studies have found substantial evidence of an obesity paradox, in which being overweight or obese leads to a better prognosis for HF patients than being normal or underweight (Valentova et al. 2016, Carbone et al. 2017). Several factors were assumed to be responsible for how this obesity paradox occurred, with cachexia in HF patients being the most plausible (Carbone et al. 2017). Cardiac cachexia is a condition that is exacerbated by cardiac obesity.

Cachexia is a metabolic syndrome characterized by involuntary and severe loss of edema-free muscle mass, which may or may not be accompanied by a reduction in fat mass. It appears following chronic diseases such as cancer and other inflammatory conditions (such as heart failure). Clinical manifestations of cachexia include skeletal muscle wasting, anemia, anorexia, and altered immune function, all of which contribute to fatigue, decreased quality of life, and decreased survival (Pureza & Florea 2013).

Cachexia includes rare conditions in which fat accumulation becomes protective rather than a risk factor (Selthofer- Relatić et al. 2019). Cardiovascular visceral obesity refers to fat accumulation in the heart, which includes intramyocardial fat, epicardial adipose tissue, and cardiac steatosis. It has been associated to the development of ischemic cardiomyopathy, cardiac microcirculatory dys-function, hypertension, atrial fibrillation, athero-sclerosis, and diabetic cardiomyopathy, but it reduces the risk of cardiac cachexia in heart failure (Soto et al. 2022).

Cachexia is diagnosed when there is a 5% weight loss in <12 months or a a body mass index (BMI) of <20 kg/m2, an underlying chronic disease, and at least three of five other criteria are met (Farkas et al. 2013, Vanhoutte et al. 2016). Cachexia incidence was observed in the majority of significant diseases, including infection, cancer, heart disease, chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), and stroke (Farkas et al. 2013). The prevalence of cachexia ranged between 0.5-1% in the general population of the United States, Europe, and Japan. Cachexia happens in 5-15% heart failure patients, with 20-40% death rates each year (Haehling et al. 2016). Global epidemiological data are still scarce and uneven in many parts of the world. Given the extremely high mortality rate among HF patients with cachexia, more research and development are urgently required.

Several observational studies on the association between cachexia and mortality in HF patients have been conducted (Castillo-Martínez et al. 2012, Saboe et al. 2015, Sato et al. 2020, Song et al. 2014). However, the studies employed different diagnostic criteria to assess cachexia, and the results were varied. Therefore, this meta-analysis was carried out to conclusively summarize the association between cachexia and mortality among HF patients.

MATERIALS AND METHODS

A literature search was performed using key concepts of “cachexia”, “heart failure”, and “mortality”. The comprehensive literature search was conducted in April 2022 through four databases (PubMed, Web of Science, Scopus, and SAGE Journals) using reference searches, citation matching, and/ or other search methods in accordance with the Preferred Reported Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al. 2021). During the literature search, no limitations on language or publication year were applied. A filter for "observational study" was utilized in the Pubmed database search.

The studies were selected independently by three people, i.e., the second author with the assistance of two other capable individuals, using inclusion and exclusion criteria. Disagreements in opinion among the researchers were discussed until an agreement was reached. The PRISMA flowchart documented the study selection process and the reasons for the study selection (Page et al. 2021).

The following criteria were employed for the literature search in this study: studies with a prospective or retrospective cohort study design and full text in English or Indonesian. The selected literature investigated adult patients (over 18 years old) with a heart failure diagnosis, as well as the intervention or exposure to cachexia based on any diagnostic criteria and/ or assessment setting. The literature also included studies comparing patients with and without cachexia and studies that had mortality outcomes from any diagnostic criteria and/ or measurement setting in any follow-up period. Studies that did not discuss mortality in patients with cachexia were omitted.

The following data were extracted from the selected studies: first author, publication year, region or country, title of study, population, study design, diagnostic criteria of cachexia, mean age, sex ratio, mean BMI, follow-up period, number of samples, number and proportion of patient with cachexia, and mortality assessment results. For the seelcted cohort studies, the Newcastle-Ottawa Scale (NOS) was employed to assess the risk of bias. Each study was

required to meet three criteria (selection, comparability, and outcomes). The study's quality would be rated "good" if the NOS assessment scores were 3 or 4 stars in selection, 1 or 2 in comparability, and 2 or 3 in outcomes; "fair" if the scores were 2 stars in selection, 1 or 2 in comparability, and 2 or 3 in outcomes; or "poor" if the scores were 0 or 1 star in selection, 0 in comparability, and 0 or 1 in outcomes (Sharmin et al. 2017).

The extracted data from the selected research were summarized and narratively presented using text and tables. A meta-analysis was performed to analyze the hazard ratio (HR) for mortality outcomes in HF patients with and without cachexia, which was reported with 95% confidence intervals (CI). The pooled analytic data included HR value and lower 95% CI, or β-coefficient as log (HR) value and standard error (SE) (Centre for Evidence-Based Medicine 2023). A random-effects model was applied due to the high heterogeneity, which was calculated using the I^2 statistical test. It would be considered statistically significant if $p<0.05$ (Higgins et al. 2022). This meta-analysis employed the Review Manager software version 5 (Cochrane), also known as RevMan 5. The protocol of this meta-analysis has been registered in the International Prospective Register of Systematic Reviews (PROSPERO) with the title "Association between Cachexia and Mortality in Heart Failure Patients: A Systematic Review" (ID: CRD42022315507).

RESULTS

A total of 844 titles and abstracts were found, 832 through database searches and 12 through reference searches, citation matching, and/ or other search method. Following the removal of duplicates (n=172), 672 titles and abstracts were screened using the inclusion and exclusion criteria. A total of 640 titles and abstracts were eliminated as irrelevant, leaving 32 titles and abstracts for which full text was retrieved. After removing 23 studies for various reasons (listed in Figure 1), the remaining studies that met the inclusion criteria were 9 studies for the qualitative synthesis and 6 studies for the quantitative synthesis (meta-analysis). The PRISMA flowchart outlined the overall selection process and reasons for exclusion.

Among the nine investigations included, two were conducted in the United Kingdom (UK) (Sze et al. 2018), two in Japan (Sato et al. 2020, Kamisaka et al. 2021), and the others in Portugal (Araújo et al. 2011), Mexico (Castillo-Martínez et al. 2012), Indonesia (Saboe et al. 2016), South Korea (Song et al. 2014), and Poland (Sobieszek et al. 2021). Most of the studies (n=8) were prospective cohort studies, with one being a retrospective cohort study. Seven studies selected subjects with chronic HF criteria, two of which specifically indicated stable chronic HF, and two studies recruited subjects with decompensated HF and acute or aggravated HF. The total number of participants gathered from those studies was 4,697, with each study having 39-1,480 subjects. Tables 1 and 2 provide summaries of all studies covered.

Most studies recruited elderly patients (≥60 years old), with only a few pre-elderly participants (45-59 years old). Six studies found that cachexia patients were typically older, while three additional studies found the opposite. The proportion of male participants was higher in the cachexia (n=3), without cachexia (n=2), and both (n=3) groups, while one research solely recruited male patients. In eight studies, the median and mean BMI of patients with cachexia ranged from 18.2 to 28.6, making them slimmer than patients without cachexia, who had a BMI of 21.1 to 30.4. Six of the eight studies found that patients with cachexia had a lower BMI than those without cachexia.

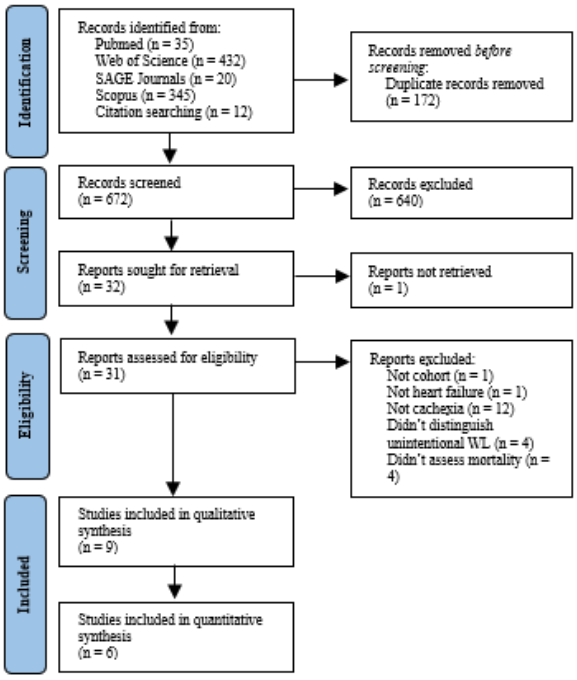

Figure 1. Flowchart of literature selection process and reasonsfor the excluded studies.

Table 1. Characteristics of the selected studies for this meta-analysis.

First author, publication year Country	Study title Population (P) Study design	Diagnostic criteria of cachexia	Age (y/o) Gender (M/F) BMI (kg/m^2)*		Follow-up period***
			Non-cachexia	Cachexia	
Anker, 1997 London, UK	Wasting as independent risk factor for mortality in chronic heart failure P: chronic heart failure patients Prospective cohort	Non-edematous and non-intentional weight loss of more than 7.5% of the previous normal non-edematous weight over a period of at least 6 months.	60±10 88.8% -	65±12 96.4% -	688±317 days
Araújo, 2011 Porto, Portugal	Nutritional markers and prognosis in cardiac cachexia P: stable chronic heart failure patients Prospective cohort	Non-edematous and non-intentional weight loss of more than 7.5% of the previous normal non-edematous weight over a period of at least 6 months.	72±11 66% 24.8±2.8	71±14 66% 23.2±3.0	486±174 days (16.2±5.8 months)
Castillo-Martínez, 2012 Mexico City, Mexico	Cachexia assessed by bioimpedance vector analysis as a prognostic indicator in chronic stable heart failure patients P: stable chronic heart failure patients Prospective cohort	Patients with vectors out of the 95% tolerance ellipse of the reference population at the lower right quadrant on BIVA evaluation as an assessment of lean body mass.	59.6±15.8 48.6% 30.4±7.2	67.2±16.4 65.8% 26.2±5.6	870±330 days (29±11 months)
Kamisaka, 2021 Nagoya, Japan	Impact of weight loss in patients with heart failure with preserved ejection fraction: Results from the FLAGSHIP study P: acute or exacerbated heart failure patients Prospective cohort	Non-obese patients with ≥5% weight loss within 6 months from discharge	81.0 (75.0-86.0)** 49.1% 21.1 (18.9-22.7)**	81.0 (72.5-86.0)** 63.3% 21.6 (19.6-22.9)**	540 days (18 months)
Saboe, 2015 Bandung, Indonesia	Cardiac cachexia and its impact on survival in heart failure patients P: chronic heart failure patients Retrospective cohort	Basic of chronic disease and weight loss >5% in 12 months or BMI <20 kg/m2 plus at least 3 of 5 criteria: decreased muscle strength, fatigue, anorexia, low fat-free mass index, abnormal biochemistry[a]	59±9 30.3% 23.10±3.18	49±17 83.3% 18.39±0.89	180 days (6 months)
Sato, 2020 Fukushima, Japan	Prognostic factors in heart failure patients with cardiac cachexia P: decompensated heart failure patients Prospective cohort	Combination of BMI < 20 kg/m2 and at least one of the biochemical abnormalities[a]	68.0 (58.0-76.0)** 62.3% 23.4 (21.5-26.0)**	76.0 (67.0-81.0)** 48.9% 18.2 (17.2-19.1)**	1295 days
Sobieszek, 2021 Lublin, Poland	Soluble ST2 proteins in male cachectic patients with chronic heart failure P: chronic heart failure patients Prospective cohort	≥5% weight loss or more in 12 months or less due to underlying illness, plus three of the following criteria: decreased muscle strength, fatigue, anorexia, low fat-free mass index, abnormal biochemistry[a]	72±14 100% 29.61±5.0	75±12.5 100% 28.27±6.6	1800 days (60 months)
Song, 2014 Seoul, South Korea	The link of unintentional weight loss to cardiac event-free survival in patients with heart failure P: chronic heart failure patients Prospective cohort	Weight loss greater than 6% of discharge body weight within 6 months in the absence of dieting or other primary causes	59±15 64.4% 24.1±3.4	68±7 40% 23.5±4.6	360 days (12 months)
Sze, 2018 Kingston upon Hull, UK	Effect of beta-adrenergic blockade on weight changes in patients with chronic heart failure P: chronic heart failure patients Prospective cohort	Weight loss of >6% between baseline and 1 year.	Increased weight: 70 (62-77)** 71% 25.9 (22.9-29.2)** Fixed weight: 72 (64-78)** 78% 27.9 (24.9-31.1)**	Decreased weight: 73 (66-78)** 68% 28.6 (24.8-32.7)**	1876 days

[a]: elevated inflammatory markers (CRP >5.0 mg/L), anemia (Hb < 12 g/dL), and/or low serum albumin levels (< 3.2 g/dL)
*: data is presented in average and/ or percentage except for 3 studies (**) which are presented in median (interquartile range)
***: follow-up periods are presented in days with an assumption of conversion for 1 month=30 days
BMI: body mass index, JVP: jugular venous pressure, ICU: intensive care unit, ESRD: end-stage renal disease, AIDS: acquired immunodeficiency syndrome, BIVA: bio-impedance vector analysis, DM: diabetes mellitus, EF: ejection fraction

Table 2. Data from the mortality assessment of patients with and without cachexia.

Study	N	Cachexia	Non-cachexia	Mortality*		Other Mortality Assessment Results
				Cachexia	Non-cachexia	
Anker, 1997	171	28 (16.4%)	143 (83.6%)	14 (50%)	35 (24.5%)	Cachexia is a predictor for all-cause mortality (HR=3.73, 95% CI: 1.93–7.23, P=0.0003)
Araújo, 2011	94	38 (40.4%)	56 (59.6%)	15 (39.4%)	6 (10.7%)	–
Castillo-Martínez, 2012	519	196 (37.8%)	323 (62.2%)	39 (19.9%)	38 (11.7%)	BIVA-cachexia is an independent predictor for mortality (β=0.504, SE=0.234, β coefficient=1.66, 95% CI: 1.05-2.62, P=0.03)
Kamisaka, 2021	452	49 (10.8%)	403 (89.2%)	17 (34.7%)	9 (2.2%)	Cachexia was a risk factor for all-cause death (HR=4.85, 95% CI: 2.15-10.81, P<0.01)
Saboe, 2015	39	6 (15.4%)	33 (84.6%)	5 (83.3%)	9 (27.3%)	Cardiac cachexia is an independent predictor for mortality (HR=50.95, 95% CI: 6.98 – 372.08, P<0.001)
Sato, 2020	1608	176 (10.9%)	1432 (89.1%)	98 (55.7%)	321 (22.4%)	Cardiac cachexia predicts all-cause mortality (HR=3.246, 95% CI: 2.587–4.071, P<0.001)
Sobieszek, 2021	91	40 (44%)	51 (56%)	19 (47.5%)	12 (23.5%)	Patients with cachexia and unfavorable factors have mortality risk for almost 7-fold higher (HR=6.89, P<0.001)
Total	2974	533	2441	207 (38.8%)	430 (17.6%)	
				637 (21.4%)		
Song, 2014	243	35 (14.4%)	208 (85.6%)	20 (8.2%)		Cachexia predicted cardiac-related death and re-hospitalization (HR=3.17, 95% CI: 1.84-5.45, P<0.001)
Sze, 2018	1480	185 (12.5%)	1295 (87.5%)	894 (60%)		Cachexia is an independent predictor for all-cause mortality (HR=1.62, 95% CI: 1.30-2.02, P<0,001)
Total	4697	753 (16.0%)	3944 (84.0%)	1551 (33.0%)		

*Mortality percentage was calculated independently by each group (cachexia and non-cachexia)
N: number of samples, HR: hazard ratio, CI: confidence interval, P: Pearson value, SE: standard error

Table 3. Risk of bias assessment of the selected studies.

	Exposure					Comparability		Outcome				
	Representativeness of the exposed cohort (*)	Selection of the non-exposed cohort (*)	Ascertainment of exposure (*)	Outcome of interest was not present at start of study[a] (*)	Total (*****)	Comparability of cohorts on the basis of the design or analysis (**)	Total (**)	Assessment of outcome (*)	Was follow-up long enough for outcomes to occur (*)	Adequacy of follow up of cohorts (*)	Total (***)	Quality score
Anker, 1997	*	*	*	-	***	*	*	*	*	*	***	Good
Araújo, 2011	*	*	*	-	***	-	-	*	*	*	***	Poor
Castillo-Martínez, 2012	*	*	*	-	***	*	*	*	*	-	**	Good
Kamisaka, 2021	*	*	*	-	***	**	**	*	*	*	***	Good
Saboe, 2015	*	*	*	-	***	**	**	*	*	-	**	Good
Sato, 2020	*	*	*	-	***	**	**	*	*	-	**	Good
Sobieszek, 2021	*	*	*	-	***	*	*	*	*	-	**	Good
Song, 2014	*	*	*	-	***	**	**	*	*	*	***	Good
Sze, 2018	*	*	*	-	***	-	-	*	*	-	**	Poor

[a]In the case of mortality studies, outcome of interest is still the presence of a disease/incident, rather than death. That is to say that a statement of no history of disease or incident earns a star.

Table 1 summarizes the various diagnostic criteria employed to assess cachexia in the studies. The total number of patients with cachexia in this meta-analysis was 753 (16.0%) out of 4697 patients, with each study having 6-196 (10.8-44%) patients (Table 2). In each study, follow-up was carried out over a 180-1,876-day period. During those periods, 1,551 (33.0%) of the 4,697 patients died. Seven studies reported that the mortality rates were 38.8% (n=207/533) in patients with cachexia and 17.6% (n=430/2,441) in patients without cachexia. It indicated that mortality was higher among patients with cachexia than those without cachexia.

Six studies in the pooled analysis showed that cachexia could significantly predict mortality in HF patients (HR=3.84, 95% CI=2.28-6.45, P<0.00001). A significant level of heterogeneity was found (P<0.00001, I^2=88%) (Figure 2). Due to a lack of data, the three other studies were not included.

Table 3 shows the results of the risk of bias assessment. Seven studies were rated as good quality, while two others were rated as poor quality. In the "exposure" category, no study received full stars since the "outcome of interest was not present at the start of the study" criteria could not be met. In the case of a mortality study, a statement of no history of disease or incident earns a star since the outcome of interest is still the presence of a disease, rather than death.

The studies were divided into two subgroups: weight loss studies (n=3) and low/lean BMI studies (n=2). Both subgroups had lower significance and heterogeneity values than the pooled analysis results. Low/lean BMI predicts a higher mortality risk than weight loss in HF patients with cachexia, with HR values of 3.96 (2.49-6.30) and 2.87 (1.35-6.09), respectively. The difference between the two subgroups was not significant (P=0.48, I^2: 0%), as demonstrated in Figure 3.

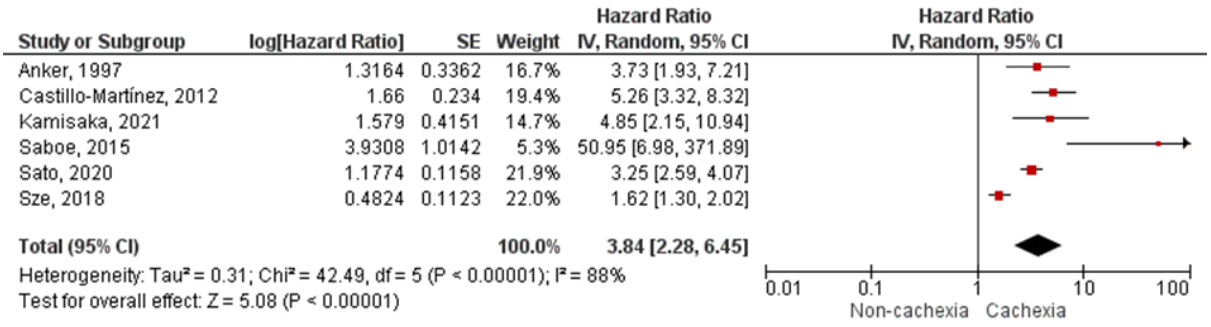

Figure 2. Hazard ratio of mortality in heart failure patients with cachexia.

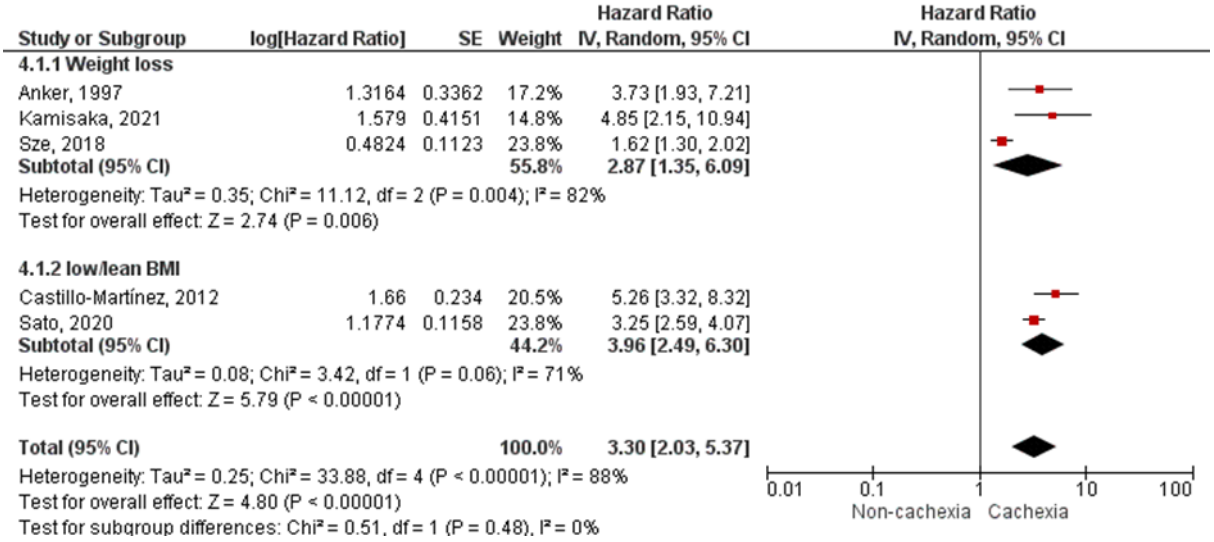

Figure 3. Analysis of mortality in the subgroups of heart failure patients with cachexia.

DISCUSSION

The prevalence of cachexia in HF patients was found to be slightly higher in this meta-analysis than in earlier studies (Haehling et al. 2016). In this meta-analysis, patients with cachexia had a lower BMI than patients without cachexia. This finding was consistent with prior research suggesting that a low BMI played a role in the diagnosis of cachexia. On the other hand, another study found higher BMI in cachexia patients (Sze et al. 2018), which is consistent with prior research that found weight loss occured more frequently in HF patients with higher BMI or obesity (Trullàs et al. 2013, Zamora et al. 2016). According to the "obesity paradox" theory, fat mass is a protective factor in HF patients, and obese patients may have higher metabolic reserve to better endure the catabolic state of HF (Zamora et al. 2016, Hamzeh et al. 2017, Krysztofiak et al. 2020). A study found that elevated BMI had no effect on the mortality and readmission of HF patients (Lestari et al. 2017). The presence of unintentional weight loss in cachexia, when the fat mass was primarily adipose tissue, might lead to a poor prognosis for HF patients with obesity (Melenovsky et al. 2013, Zamora et al. 2016, Valentova et al. 2020).

Although the mortality rate in patients with cachexia was lower than in those without cachexia, it should be noted that the number of HF patients with cachexia was also lower than in the overall population. Cachexia served as an independent mortality predictor, indicating a greater mortality rate as compared to when cachexia was absent. This meta-analysis found that cachexia had a nearly four-times worse prognosis in HF patients, which is consistent with prior studies. This signifies that HF patients with cachexia were nearly four times more likely to die than those without cachexia at any given time. HF patients diagnosed with cachexia based on low/lean BMI had a higher mortality risk than those diagnosed based on weight loss. However, no significant difference was observed in this meta-analysis between diagnosing cachexia by weight loss or low/lean BMI, indicating that both weight loss and low/lean BMI were relevant as the diagnostic criteria of cachexia.

According to the European Society of Cardiology (ESC) Guidelines, one of the comorbidities of HF is

cachexia, which can be caused by many factors, including catabolic-anabolic imbalances, neuro-hormonal disorders, pro-inflammatory cytokine activation, anorexia, malabsorption, and anabolic hormone resistance (Sandek et al. 2014, Okoshi et al. 2014, Valentova et al. 2016, Raposo André et al. 2017). Although it is unclear if cachexia directly causes death in HF patients, multiple observational studies have shown that cachexia indeed played a role in HF patients' poor prognosis.

Kalantar et al. (2013) found that elevated platelet count and activation, as well as arrhythmias, appeared to be the most likely contributors to the occurrence of sudden cardiovascular events, including death, in cachexia patients. Patients with cachexia might develop atherosclerotic plaques, which can increase the risk of developing acute coronary syndrome and/ or death due to sudden cardiac diseases, as a result of increased platelet count and activation accompanied by endothelial dysfunction and increased pro-inflammatory cytokines (Molnar et al. 2011, Fatkhullina et al. 2016, Anaszewicz & Budzyński 2017, Amalia et al. 2022). They could be more prone to developing arrhythmias, particularly atrial fibrillation (Arámbula-Garza et al. 2016, Anaszewicz & Budzyński 2017). Platelet-to-lymphocyte ratio (PLR) can be a supplementary biomarker for symptomatic HF, particularly in patients with acute coronary syndrome (ACS) (Intan et al. 2022). A study discovered that patients with tetralogy of Fallot (TOF), a congenital abnormality that interferes with proper cardiac blood flow, had a low BMI below the normal limit (Saputri et al. 2020). Furthermore, muscle loss in cachexia patients might lead to a decrease in cardiac contractility, which worsens the HF condition in these patients (Fulster et al. 2013, Drescher et al. 2015.

Strength and limitations

Recent studies to assess cachexia have employed various diagnostic criteria, resulting in varied outcomes. This meta-analysis has conclusively summarized the association between cachexia and mortality among HF patients. Several limitations should be noted in evaluating the results of this meta-analysis. First, the scarcity of research and the incompleteness of data from the selected studies. Second, it is important to understand and take into consideration that the mortality outcome employed in this meta-analysis was all-cause mortality. This suggests that mortality in the recruited patients was not necessarily caused by cachexia and/or HF, but could possibly be caused by other diseases or comorbidities, e.g., infection, diabetes, COPD, and kidney failure. Third, the pooled analysis revealed results with significant heterogeneity, most likely due to variations in the number of samples, diagnostic criteria, and length of follow-up period between studies.

CONCLUSION

Cachexia worsens the prognosis of heart failure patients. Mortality rates were higher among heart failure patients with cachexia compared to those without cachexia. Medical professionals should pay more attention to heart failure patients' nutritional state and cardiac cachexia in order to not worsen their prognosis.

Acknowledgment

The authors would like to thank fellow students from the Medical Study Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. The authors would like to extend their gratitude to Caesaroy Afif Wibowo and Brigita Deya Widiana for helping with the literature screening and selection.

Conflict of interest

None.

Funding disclosure

None.

Author contribution

AN conceptualized and supervised the study, as well as validated, reviewed, and finalized the manuscript. UN conceptualized the study, managed the administration, collected and analyzed the data, and wrote the manuscript. SW and MA validated and supervised the study, as well as reviewed and finalized the manuscript. HO validated, reviewed, and finalized the manuscript.

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Meta-Analysis

FIVE SINGLE-NUCLEOTIDE POLYMORPHISMS IN THE PITX2 GENE AS RISK FACTORS FOR ATRIAL FIBRILLATION

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ABSTRACT

Atrial fibrillation (AF) is a highly prevalent arrhythmia. The involvement of molecular mechanisms in increased AF risk remains uncertain. However, the paired-like homeodomain transcription factor 2 or pituitary homeobox 2 (PITX2) gene has been linked to AF development. A comprehensive search was carried out to identify all eligible case-control studies in order to assess the association between five single-nucleotide polymorphisms (SNPs) in the PITX2 gene and the risk of AF. This meta-analysis employed the Review Manager (RevMan) software version 5.3 (Cochrane). There were 13 clinical studies, with a total of 11,961 subjects, that met the inclusion criteria. These subjects consisted of 4,440 patients with AF and 7,521 controls. The meta-analysis of five SNP types in the PITX2 gene was done using crude odds ratios (ORs). This revealed that rs2200733 increased the risk of AF (OR=1.80; 95% CI=1.53-2.11; p=0.0005; I^2=80%). On the other hand, the other three SNPs decreased the risk of AF, namely, rs385344 (OR=0.75; 95% CI=0.59-0.95; p=0.002; I^2=85%), rs6838973 (OR=0.64; 95% CI=0.51-0.81; p=0.0001; I^2=73%), and rs17570669 (OR=0.80; 95% CI=0.65-0.98; p=0.03; I^2=70%). However, there was no significant association between rs10033464 and AF (OR=1.21; 95% CI=0.97-1.50; p=0.13; I^2=83%). In conclusion, depending on the type, SNPs in the PITX2 gene correlate with AF risk factors, either by alleviating or reducing the risk.

Keywords: Atrial fibrillation; pituitary homeobox 2 (PITX2) gene; chromosome 4q25; single-nucleotide polymorphisms (SNPs); cardiovascular diseases

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Article history
●Submitted 22 Dec 2022 ● Revised 27 Jan 2023 ● Accepted 10 Feb 2023 ● Published 10 Mar 2023

How to cite: Putra RM, Dharmadjati BB, Pikir BS, et al (2023). Five Single-Nucleotide Polymorphisms in the PITX2 Gene as Risk Factors for Atrial Fibrillation. Folia Medica Indonesiana, 59 (1), 85-91.
https://doi.org/10.20473/fmi.v59i1.37126

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. A genetic variable has been identified as an atrial fibrillation risk factor.
2. Rs2200733 is a type of SNP that increases atrial fibrillation risk, whereas rs3853445, rs6838973, and rs17570669 have the reverse effect.

INTRODUCTION

The global atrial fibrillation (AF) prevalence is rising. Data from the United States (US) revealed that approximately two million people suffer from AF, with the number anticipated to rise to six to ten million by 2050 (Morillo et al. 2017). Over 454,000 persons in the US were hospitalized with AF as the primary diagnosis (Benjamin et al. 2019). In Indonesia, the AF prevalence has risen from 7.1% in 2010 to 9.0% in 2011, 9.3% in 2012, and 9.8% in 2013 (Hutomo & Subagjo 2020).

AF increases the risk of stroke by two to three times, and can also increase the risk of heart failure (Anter et al. 2009, Kamel et al. 2016, Alsagaff et al. 2022). Identifying and screening for AF risk factors may help to prevent future disease and complications. There are numerous risk factors for AF, including hypertension, heart valve disease, and unmodifiable

risk factors (e.g., genetic factors) (Staerk et al. 2017). In the last five years, there has been an increase in genetic study on the prevalence of AF, and various loci have been discovered to have a role in the occurrence of AF (Kornej et al. 2020).

Several studies have found that a gene on the chromosome 4q25 has a significant role in the incidence of AF. One of the largest studies on genetic factors and AF, conducted in Iceland in 2007, indicated that the single-nucleotide polymorphisms (SNPs) rs2200733 and rs10033464 enhanced the incidence of AF. According to studies involving 500 AF and lone AF patients and a control group of 4,476 patients, SNPs rs385344, rs6838973, and rs17570669 affected the onset of AF, notably at loci with minor alleles (Gudbjartsson et al. 2007, Lubitz et al. 2010, Arndt & MacRae 2014). These variants are found around the paired-like homeodomain transcription factor 2 or pituitary homeobox 2 (PITX2) transcription factor gene, which encodes for a homeobox transcription factor (Kirchhof et al. 2011). These are essential for the formation of sinoatrial nodes and left-right asymmetry of the heart (Franco et al. 2017). PITX2 isoform deletion results in congenital malformations, conduction system problems, and myocardial defects (Yuan et al. 2013, Zhao et al. 2015). Its deletion also shortens the duration of the atrial action potential, making AF more susceptible (Bai et al. 2020). In recent five years, there has been no meta-analysis study that discusses the association between the PITX2 gene and the occurrence of AF in diverse populations. This meta-analysis investigated various SNPs in the PITX2 gene of chromosome 4q25 that are correlated with the occurrence of AF.

MATERIALS AND METHODS

A systematic literature search was conducted in this meta-analysis to identify all case-control or prospective cohort studies that investigated the association of five types of SNP (rs2200733, rs10033464, rs385344, rs6838973, rs17570669) in the PITX2 gene against the risk of AF and lone AF. The literature search utilized PubMed, ScienceDirect, ProQuest, EBSCO, Springer, and Cochrane databases, using the following keywords: "gene polymorphism" or "SNP" or "single nucleotide polymorphism" and "atrial fibrillation" or "AF" or "lone AF ".

The main criteria in this meta-analysis were all studies that described the association between the five types of SNP and the occurrence of AF. Several additional inclusion criteria were implemented in the literature search: (1) studies investigating patients with AF or lone AF; (2) prospective cohort or case-control studies; (3) studies using odd ratios (OR) or relative risk parameters (RR), with 95% confidence intervals; (4) studies utilizing independent variables in the form of one or more than five SNPs (rs2200733, rs10033464, rs385344, rs6838973, rs17570669). The collected data were described in a specific format that met the standards for meta-analysis, then the data were classified based on the following criteria: the name of the main author, publication year, country of publication, study design, gender composition, age range, average age, allele frequency, and genotype distribution in each case and control group (Mohanty et al. 2013).

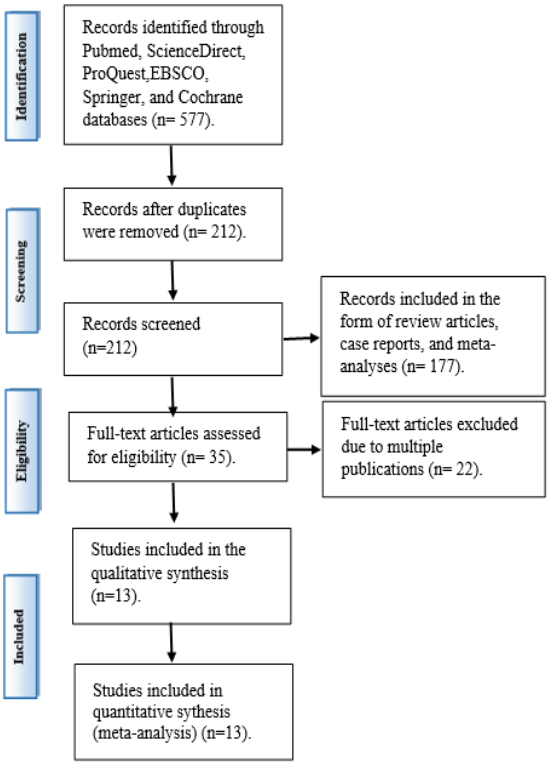


Figure 1. Steps in eliminating studies and extracting data.

A bias risk assessment was performed using the Newcastle–Ottawa Scale (NOS) for cohort and case control studies. The NOS cover four domains, which include selection, comparability, and exposure (Lo et al. 2014). Each question in the comparability domain could be given a "0" star if it is not contemplated, a "1" if the item is contemplated, and a maximum star of "2". The total number of stars represents the quality of the study, which can be interpreted as poor quality (0-2 stars), fair quality (3-5 stars), or good/high quality (6-9 stars).

The Chi-square test was used to assess the Equilibrium Hardy-Weinberg (EHW) in control group. Data recapitulation and analysis were carried out using Software Review Manager (Rev.Man) 5.0

and Comprehensive Meta-Analysis Software 3.3. The data heterogeneity was assessed using the Q statistic and the I^2 statistic, with a significance (p-value) less than 0.05, as suggested by Smith et al. (2012).

RESULTS

The initial search yielded 577 scientific articles. In the screening process, 212 articles were identified as potentially relevant to this meta-analysis. However, 22 items were duplicates and 177 items were removed because they included 135 review articles, 38 case reports, and 4 meta-analysis articles. As a result, thirteen articles that matched the inclusion criteria for this meta-analysis were obtained.

As shown in Table 1, eleven studies, including a total of 8,388 cases and 13,526 controls, examined the association of SNP rs2200733 with the risk of AF. A study by Mohanty et al. (2013) revealed a strong correlation between rs2200733 and AF occurrences, with a p-value of 0.006. The prevalence of the recessive allele (T allele) in patients with AF was 26.9%, whereas it was 14.5% in controls. Furthermore, Kalinderi et al. (2015) discovered that the T allele was more common in AF patients. A significant association between rs2200733 and AF events was also reported in studies by Lee et al. (2010), Lubitz et al. (2010), Olesen et al. (2012), Kolek et al. (2014), Kiliszek et al. (2016), and Zhao et al (2017).

However, according to Bhanushali et al. (2017), rs2200733 was not related with AF. A similar finding was found in a study by Henningsen (2010). When these eleven studies were statistically

Table 1. Association between SNP rs2200733 and AF.

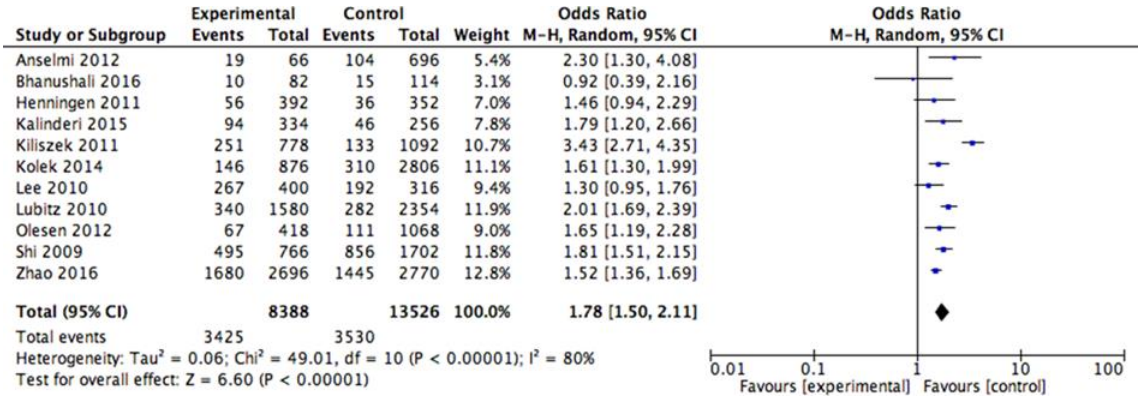


Table 2. Association between SNP rs10033464 and AF.

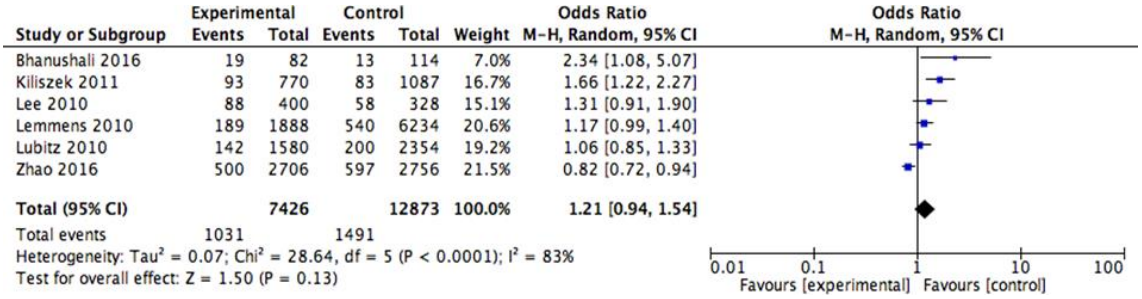
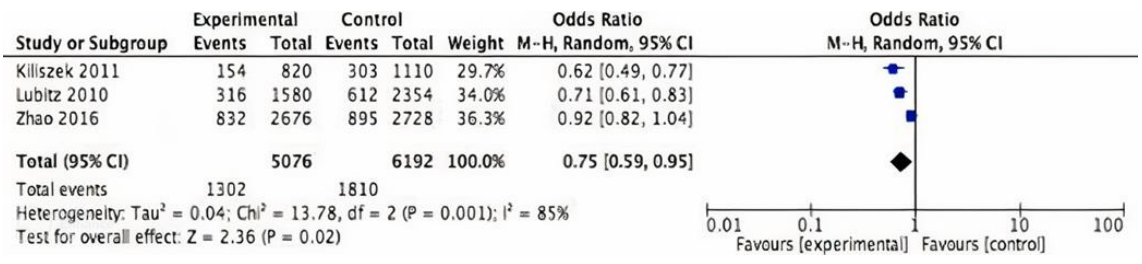


Table 3. Association between SNP rs3853445 and AF.



analyzed, there was a positive correlation between SNP rs2200733 and the prevalence of AF (OR=1.78, 95% CI=1.5-2.11, p<0.00001). It implies that subjects with recessive alleles (T) were more likely to experience AF episodes than subjects with dominant alleles (C).

Six studies with 7,426 cases and 12,873 controls were analyzed, as seen in Table 2. According to Bhanushali et al. (2017), there was a substantial connection between rs10033464 (T allele) with AF occurrences when compared to a control group. Kiliszek et al. (2016) also found that rs10033464 was related to an increased risk of AF. Zhao et al. (2017) obtained comparable results to the previous research. However, Lee et al. (2010) found that rs10033464 was not significantly associated to the occurrence of AF in their study. A similar notion was also stated by Lubitz et al. (2010). The analysis of these six studies revealed no significant correlation between SNP rs 10033464 and the risk of AF (OR=1.21, 95% CI=0.94-1.54, p=0.13, I^2=83%).

There were only three studies investigating rs3853445 and AF, with a total of 5,076 cases and 6,192 controls (Table 3). A study by Kiliszek et al. (2016) did not show any significant correlation between rs3853445 and AF. Conversely, Lubitz et al. (2010) discovered that a minor allele of SNP rs3853445 was associated with a decreased risk of AF. The analysis discovered a significant negative correlation between SNPrs3853445 and the risk of AF (OR=0.75, 95% CI=0.59-0.95, p=0.2).

Only two of the selected studies investigated the association between rs6838973 and AF, with a total of 807 cases and 1,492 controls (Table 4). Kiliszek et al. (2016) reported that rs6838973 had a substantial protective effect against AF. Lubitz et al (2010) reached a similar conclusion in their study. A statistical analysis was performed, and it was discovered that there was a considerably negative correlation between SNP rs6838973 and the probability of AF (OR=0.64, 95% CI=0.59-0.81, p=0.0001).

Table 5 shows that only two of the selected studies reported on rs17570669 and AF, with a total of 157 cases and 279 controls. According to the findings of a study by Kiliszek et al. (2016), rs17570669 had a protective effect against AF. In a study by Lubitz et al. (2010), rs17570669 was associated with a lower risk of AF after an additional adjustment of the rs2200733 genotype. A significant negative correlation was found between SNP rs17570669 and the likelihood of AF incidence (OR=0.80, 95% CI=0.65-0.98, p=0.03).

DISCUSSION

A meta-analysis of five types of SNP (rs2200733, rs10033464, rs385344, rs6838973, rs17570669) found in the PITX2 gene of chromosome 4q25 was conducted in this study. The PITX2 gene, also known as the paired-like homeodomain transcription factor 2 or pituitary homeobox 2 (PITX2), is located on chromosome 4q25. This gene is involved in the formation of cardiac asymmetry. Damage to this gene was found to play a role in the development of congenital defects, such as atrial isomerism, double outlet right ventricle (DORV), aortic arch anomalies, and malignant right coronary artery (Henningsen et al. 2010, Purwowiyoto & Surya 2021). The PITX2 gene also served a role in AF, according to a genome-

Table 4. Association between SNP rs6838973 and AF.

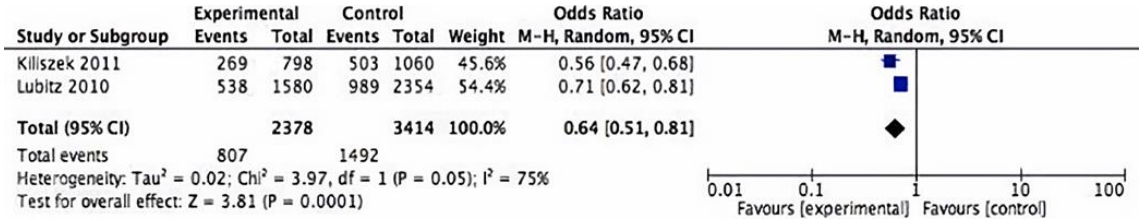
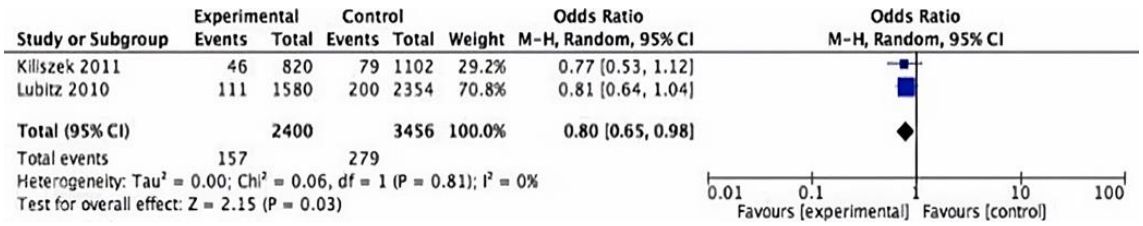


Table 5. Association between SNP rs17570669 and AF.



wide association study (GWAS) conducted in 2007. A study conducted on 550 patients and 4,476 controls discovered that SNP rs22000733 in the PITX-2 gene was involved in the incidence of AF. Following this study, another investigation focusing on the association between SNP and the occurrence of AF discovered some other SNPs, including rs10033464, rs385344, rs6838973, and rs17570669, which were predicted to have a link with the occurrence of AF (Lubitz et al. 2010, Schnabel et al. 2011, Bhanushali et al. 2017).

The conclusion of this meta-analysis showed that the roles of SNPs in the incidence of AF differed from each other. A meta-analysis conducted in 2012 indicated that the existence of minor alleles in SNP rs22000733 had a positive correlation with AF among the majority of AF patients in Europe (OR=1.89). This is remarkably comparable to the results obtained from this study, with the OR for the incidence of AF being 1.78. However, the population in this meta-analysis was more diverse than the sample in the 2012 meta-analysis. From the eleven selected studies, three were conducted in East Asia, one in Central Asia (India), four in Europe, and three in America. Diverse populations might lead to consistent results, which found that SNPs had significant roles in the incidence of AF (Smith et al. 2012). On the other hand, Bhanushali et al. (2017) determined that SNP rs22000733 had no significant correlation to theincidence of AF (OR=0.92). This could happen, however, because the number of samples used was only 25, consisting of 10 AF patients and 15 controls. In studies with hundreds or thousands of samples, such as those conducted by Zhao et al. (2017), Kolek et al. (2014), Kalinderi et al. (2015), SNP rs22000733 consistently demonstrated a positive correlation with the incidence of AF.

A meta-analysis of SNPs rs3853445, rs6838973, and rs17570669 found that minor alleles in the three SNPs had a protective effect against the occurrence of AF. Kiliszek et al. (2016) and Lubitz et al. (2010) conducted two major investigations that had a significant impact in this result. Kiliszek et al. (2016) conducted a study in Poland with 400 AF cases and 550 controls. Several novel SNPs (i.e., rs3853445, rs6838973, and rs17570669) in the PITX2 gene were investigated for their association with the occurrence of AF. Lubitz et al. (2010) observed in a prior study that the three SNPs listed above showed a negative correlation with the occurrence of AF. The study had discovered no significant relation between SNP and the occurrence of AF due to the smaller sample size. To draw conclusions on the protective effects of SNP on AF, studies with bigger sample sizes are required.

Finally, SNP rs10033464 was discovered to have no statistically significant relationship with the prevalence of AF. However, the findings of some of the studies included in this meta-analysis were not consistent. Among these studies were those by Bhanushali et al. (2017), who reported a positive correlation of the SNP to the occurrence of AF (OR=2.34), and Lemmens et al. (2010) who only identified a minor correlation. Other studies, on the other hand, discovered a negative association between the SNP and the occurrence of AF (OR=0.80), as well as an insignificant impact of the SNP on the risk of AF (Lee et al. 2010, Zhao et al. 2017). The discrepancies in the results among the selected studies might be attributed to the ethnicity of the subjects involved. Genetic information contains historical, anthropological, and statistical characteristics that vary across ethnicities (Sulistyorini et al. 2017, Yudianto et al. 2017). According to research conducted by Bhanushali et al. (2017), minor allele frequency was more common in Utah (US) residents of Northern and Western European ancestry, as well as Gujarati Indians in Houston (US), whereas allelic frequency differences were more common in Han Chinese in Beijing (China), Yoruba in Ibadan (Nigeria), and Japanese in Tokyo (Japan).

Several investigations on the relevance of genetic polymorphism in the occurrence of AF have indicated a significant association. However, the mechanism of how these genes affect the pathophysiology of AF is unknown (Olesen et al. 2012). An animal study demonstrated that animals with a PITX2 deficiency did not develop pulmonary myocardial sleeves (Chinchilla et al. 2011). Clinical research revealed that the sleeves induced ectopic beats, which play a crucial role in the atrial fibrillation process (Stavrakis et al. 2015). This mechanism is connected to AF ablation, which entails the electrical isolation of those sleeves in the pulmonary veins (Maesen et al. 2016). A better understanding of AF in general could improve the quality of cardiovascular disease healthcare services, such as the classification of AF using new methods (Yazid & Rahman 2020).

Strength and limitations

Understanding the genetic polymorphisms that contribute to the risk factor of AF could accelerate healthcare-seeking behavior and a reduction in AF mortality. The purpose of this meta-analysis was also to identify five SNPs as factors that could affect the risk of AF. This meta-analysis covered numerous studies with subjects from a wide range of ethnicities. However, the heterogeneous study sample might have caused variability in the obtained data.

CONCLUSION

This present meta-analysis identified five types of SNP as risk factors for atrial fibrillation (AF) across several studies and demographics. Individuals carrying rs2200733 are at a higher risk of developing AF. Conversely, rs3853445, rs6838973, and rs17570669 demonstrated a protective effect against AF.

Acknowledgment

The authors would like to thank all the seniors, fellows, and staff at the Faculty of Medicine, Universitas Airlangga, as well as the Department of Cardiology and Vascular Medicine at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, for their assistance in carrying out this study.

Conflict of interest

None.

Funding disclosure

None.

Author contribution

RP, IM, and II designed the study, gathered the data, and conducted the data analysis. RP and JA interpreted the data for the study and drafted the manuscript. BD and BP revised the manuscript for important intellectual content and approved the final version for publication.

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Macdonald S. editor. Maye's midwifery 14th ed. [eBook]. Edinburgh: Bailliere Tindall; 2011 [cited 2012 Aug 26]. Available from: Ebrary.

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Offline proceeding

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Pahl KM. Preventing anxiety and promoting social and emotional strength in early childhood: an investigation of risk factors [dissertation on the Internet]. St Lucia, Qld: University of Queensland; 2009 [cited 2017 Nov 22]. Available from: https://espace.library.uq.edu.au/view/UQ:178027.

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Cancer-Pain.org (2002). New York: Association of Cancer Online Resources, Inc.; c2000-01. [updated 2002 May 16]. Available from http://www.cancer-pain.org/. Accessed July 9, 2002.

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