Vol.59 No.4 [12/2023]

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.4 [12/2023]

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

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FOLIA MEDICA INDONESIANA
p-ISSN 2355-8393, e-ISSN 2599-056X
Vol.59 No.4 [12/2023]

Original Research Report

Systematic Review

FOLIA MEDICA INDONESIANA

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

Vol.59 No.4 [12/2023]

Original Research Report

HYPERTENSION AND LIPID PROFILES IN MIDDLE-AGED MALE PATIENTS: A STUDY AT A TERTIARY HOSPITAL IN SURABAYA, INDONESIA

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ABSTRACT

Hypertension is one of the most frequent non-communicable diseases and a risk factor for cardiovascular disease, contributing to most deaths globally. The risk of hypertension is higher in those with uncontrolled lipids. In this study, the incidence of hypertension and lipid profiles were examined from March 2020 to March 2022. The aim of this study was to determine the relationship between hypertension in men aged 45–60 years and their lipid profiles, which include total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). This study used an analytical observational design with a cross-sectional approach. Medical records were utilized as secondary data. Statistical analysis was conducted using the Spearman rank correlation test. Statistical significance was determined at $p<0.05$. This research examined 115 patients with hypertension. The results showed that the stage of hypertension was correlated with total cholesterol ($r=0.317$; $p=0.001$) and triglyceride levels ($r=0.217$; $p=0.02$). However, the stage of hypertension was not significantly correlated with LDL ($r=0.158$; $p=0.91$) and HDL ($r=0.75$; $p=0.423$). Hence, this current study underscores the nuanced relationship between lipid profiles and the stage of hypertension in middle-aged male patients. This study highlights the importance of sex-specific analysis in hypertensive research. It also provides promising avenues for further investigation.

Keywords: Cardiovascular disease; hypertension; triglycerides; low-density lipoprotein (LDL); high-density lipoprotein (HDL)

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Article history
●Submitted 13/09/2023 ● Revised 28/10/2023 ● Accepted 27/11/2023 ● Published 10/12/2023

How to cite: Nurtya A, Tjempakasari A, Maimunah U, et al (2023). Hypertension and Lipid Profiles in Middle-Aged Male Patients: A Study at a Tertiary Hospital in Surabaya, Indonesia. Folia Medica Indonesiana 59 (4), 321-328, https://doi.org/10.20473/fmi.v59i4.49674

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

Highlights:
1. Uncertainty regarding the association between hypertension and the levels of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) signifies the need for more research, particularly in the realm of sex-specific analysis.
2. Our study uncovered significant associations between hypertension and the levels of total cholesterol and triglycerides, expanding our understanding of the impact of lipid profiles on hypertension.
3. This research may potentially pave the way for personalized diagnostic and therapeutic strategies, ultimately enhancing hypertension management and patient outcomes.

INTRODUCTION

Hypertension poses a unique challenge to the population of Surabaya, Indonesia. According to the Eighth Joint National Committee (JNC VIII) guidelines, hypertension is characterized by systolic or diastolic blood pressure exceeding 140/90 mmHg. This condition poses a significant threat to cardiovascular health and is a major contributor to global mortality. Hypertension is broadly classified into two categories: primary hypertension, which lacks a specific underlying cause and accounts for approximately 90% of cases, and secondary hypertension, which is linked to underlying medical conditions such as renal, vascular, thyroid, or adrenal diseases (James et al. 2014, Putri et al.

2021).

The prevalence of hypertension is a global concern, affecting an estimated 1.13 billion people worldwide. This number is projected to increase to 1.5 billion by 2025 (Kingue et al. 2015, Nawi et al. 2021). The hypertension epidemic is a pressing public health concern because it particularly affects Southeast Asian populations, including those in Indonesia. Alarmingly, the Indonesian Ministry of Health data indicated that only 32.2% of hypertensive individuals in Indonesia have been diagnosed and treated. Their recent Basic Health Research report revealed a troubling increase in hypertension prevalence, rising from 25.8% in 2013 to 34.11% in 2018. East Java Province is of particular concern, with a hypertension rate of 36.32%. Surabaya, the province's capital city, revealed a prevalence of 31.58% (Minister of Health of the Republic of Indonesia 2013, 2018). The specifics of what differentiates the population in Surabaya from other regions warrant further research and analysis. Factors such as lifestyle, dietary habits, genetics, access to healthcare, and environmental influences may play a role in the higher prevalence of hypertension in this area. It is essential to understand these distinctive elements for the development of targeted public health interventions and policies to address the challenges of hypertension management in Surabaya and its surrounding areas.

An increased risk of hypertension has been associated with dyslipidemia. This imbalance of lipids is defined by elevated total cholesterol, low-density lipoprotein (LDL), and triglyceride levels together with lower high-density lipoprotein (HDL) levels (Islam et al. 2014, Astana & Triyono 2018). According to earlier research, excess triglycerides and lipids contribute to atheroma formation on blood vessel walls, impairing flexibility and potentially leading to hypertension. Notably, a significant portion of individuals with hypertension exhibit moderate blood cholesterol levels of 200–239 mg/dL (Kusmiati & Pratiwi 2015, Maryati 2017).

For several reasons, it is essential to research hypertension in men, particularly in the age group of 45–60. There are notable sex-specific differences in how hypertension presents, with men exhibiting a higher prevalence compared to women. The investigation of hypertension in men allows for the identification of distinct risk factors, etiological factors, and health implications. Age-specific variations in hypertension have also been documented, with the risk increasing with age. Focusing on this age group allows for a better understanding of age-related patterns and correlations, leading to more targeted preventive measures and interventions. Additionally, given the significant health concern of hypertension in older men, this study may help uncover the factors contributing to its high prevalence by offering insights to guide public health efforts. Lastly, understanding sex and age-specific differences enables the customization of preventive strategies and interventions through lifestyle modifications, targeted screening, or specialized treatments tailored to the needs of this specific population (Choi et al. 2017). In summary, this research is crucial for gaining insights into the unique factors contributing to hypertension in middle-aged men and, in turn, providing information on effective strategies for its prevention, diagnosis, and management.

Numerous studies have reported age- and sex-specific variations in hypertension. However, comprehensive research on the relationship between lipid profiles and hypertension, especially among men aged 45–60 in the Surabaya area, remains scarce. Consequently, further investigations are warranted to elucidate this connection in an endeavor to reduce hypertension prevalence and promote public health awareness. This research aimed to determine the relationship between lipid profiles (i.e., total cholesterol, triglycerides, LDL, and HDL levels) and hypertension in men aged 45–60 years in the Department of Internal Medicine and Department of Cardiology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, in the period of March 2020–March 2022.

MATERIALS AND METHODS

This analytical observational research used a cross-sectional design and utilized secondary data from the medical records of hypertensive patients. The medical records were collected from patients at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, who met the specified inclusion and exclusion criteria. This study examined all medical record data of hypertensive patients admitted to the Department of Internal Medicine and Department of Cardiology in the period spanning from March 2020 to March 2022. The total samples were 115 hypertensive patients selected via non-random total sampling. The specified inclusion criteria were individuals who were diagnosed with primary hypertension, were male, and fell within the age group of 45–60 years. Meanwhile, the exclusion criteria pertained to patients with incomplete medical record data regarding their lipid profiles from the laboratory tests. The data collected included hypertension severity and lipid profiles, with data on the stage of hypertension retrieved from the initial patient records (Cuschieri 2019).

The measurement of total cholesterol, triglyceride, LDL, and HDL levels was conducted through

laboratory tests. The results of the laboratory tests were recorded in the patients' medical records. Desirable total cholesterol levels were defined as being <200 mg/dL, while levels of 200–239 mg/dL and ≥240 mg/dL were categorized as borderline high and high, respectively. The acceptable range for triglyceride levels is defined as being below 200 mg/dL. Levels from 200 to 399 mg/dL are considered as borderline high, while equal to or beyond 400 mg/dL are categorized as significantly high. The desired LDL levels are below 130 mg/dL, while between 130 and 159 mg/dL are borderline high, and equal to or above 160 mg/dL are categorized as high. Conversely, HDL levels were considered desirable if they were >50 mg/dL, borderline high if they were 40–49 mg/dL, and high if they were <40 mg/dL. The measurement of blood pressure was conducted by utilizing systolic and diastolic pressure readings, expressed in millimeters of mercury (mmHg). According to the Eighth Joint National Committee (JNC VIII) guidelines, blood pressure readings of 140–159 mmHg systolic and 90–99 mmHg diastolic were categorized as stage 1 hypertension. On the other hand, blood pressure readings of ≥160 mmHg systolic and ≥100 mmHg diastolic were categorized as stage 2 hypertension (MacLaughlin et al. 2018).

The statistical analysis was conducted using IBM SPSS Statistics for Mac, version 26.0 (IBM Corp., Armonk, N.Y., USA). The analysis began with univariate analysis, which provided a descriptive representation of the variables through frequency distribution tables. Subsequently, bivariate analysis was employed to examine the relationship between lipid profiles and the incidence of hypertension. A Kolmogorov-Smirnov test was conducted to assess the normality of the continuous variables, and the results indicated that all of the variables had a non-normal distribution ($p<0.05$). Hence, an analysis of the association between continuous variables was carried out by employing Spearman's rank correlation coefficient, which assessed the strength of the relationship between two variables on an ordinal or interval scale. The statistical significance level was established at $p<0.05$. The correlation coefficient (r) represented the strength of the association. A negative value signified an inverse correlation, while a positive value implied a linear correlation. The correlation coefficient ranged from 0.00 to 1.00, with specific intervals indicating different levels of relationship strength. The strength of the relationship was categorized into several categories, i.e., very weak (0.00–0.19), weak (0.20–0.39), moderate (0.40–0.59), strong (0.60–0.79), and very strong (0.80–1.00) (Schober & Schwarte, 2018, Aslam, 2020).

This study received ethical approval from the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, with reference No. 1062/LOE/301.4.2/X/2022 dated 6/10/2022.

RESULTS

This study included 115 subjects who met the specified inclusion and exclusion criteria. Table 1 presents the frequency distribution of patients categorized by age, initial hypertension stage, follow-up hypertension stage, and lipid profiles. Notably, 48.7% of hypertensive patients in the Department of Internal Medicine and Department of Cardiology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, fell within the age group of 56–60 years. The primary hypertension stage at the first visit was stage I, accounting for 60% of cases. During follow-up visits, the majority of hypertensive patients (54.8%) exhibited pre-hypertension. Borderline-high total cholesterol levels (43.5%) were prevalent, while borderline-high triglyceride levels were observed in most hypertensive individuals (75.7%). A significant proportion (61.7%) of the patients had desirable LDL levels, and 53% of the patients had borderline-high HDL levels.

Table 2 reveals distinct patterns in lipid profiles among patients diagnosed with stage I and stage II hypertension. The total cholesterol levels were 76% in individuals with stage I hypertension and 64.3% in individuals with stage II hypertension. Borderline-high triglyceride levels were observed in 56.3% of stage I hypertension cases and 43.7% of stage II hypertension cases. A significant proportion of patients in both categories showed desirable LDL levels, comprising 60.6% in stage I hypertension and 39.4% in stage II hypertension. Furthermore, the most frequent HDL category for both stages was borderline high, with 55.7% in stage I hypertension and 44.3% in stage II hypertension.

Table 3 presents the results of the Spearman correlation analysis, which examined the relationship between lipid profiles and hypertension. The results exhibited a significant and moderately positive correlation between total cholesterol and hypertension, with $p=0.001$ ($p<0.01$) and $r=0.317$. Triglycerides had a significant relationship with hypertension, albeit with a very weak positive correlation, with $p=0.020$ ($p<0.05$) and $r=0.217$. Conversely, there was no statistically significant correlation observed between LDL and hypertension, as evidenced by $p=0.091$ ($p>0.05$). Lastly, HDL and hypertension showed no correlation, as indicated by $p=0.423$ ($p>0.05$).

Table 1. Frequency distribution of patients according to age, the stage of hypertension, and lipid profiles.

Variables	n	%
Age (years)		
45-49	16	13.9
50-55	43	37.4
56-60	56	48.7
Initial hypertension stage		
Stage I	69	60
Stage II	46	40
Follow-up hypertension stage		
Normal	15	13.0
Pre-hypertension	63	54.8
Stage I	27	23.5
Stage II	10	8.7
Total Cholesterol (mg/dL)		
Desirable	23	20.0
Borderline high	50	43.5
High	42	36.5
Triglyceride (mg/dL)		
Desirable	26	22.6
Borderline high	87	75.7
High	2	1.7
LDL		
Desirable	71	61.7
Borderline high	33	28.7
High	11	9.6
HDL		
Desirable	11	9.6
Borderline high	61	53.0
High	43	37.4

Table 2. Data distribution of lipid profiles according to the stage of hypertension at the first visit.

Variables		The stage of Hypertension (n=115)		
		Stage I	Stage II	Total
Total Cholesterol	Desirable	16 (69.6%)	7 (30.4%)	23 (100%)
	Borderline high	38 (76%)	12 (24%)	50 (100%)
	High	15 (35.7%)	27 (64.3%)	42 (100%)
Triglyceride	Desirable	20 (76.9%)	6 (23.1%)	26 (100%)
	Borderline high	49 (56.3%)	38 (43.7%)	87 (100%)
	High	0 (0%)	2 (100%)	2 (100%)
LDL	Desirable	43 (60.6%)	28 (39.4%)	71 (100%)
	Borderline high	17 (51.5%)	16 (48.5%)	33 (100%)
	High	9 (81.8%)	2 (18.2%)	11 (100%)
HDL	Desirable	8 (72.7%)	3 (27.3%)	11 (100%)
	Borderline high	34 (55.7%)	27 (44.3%)	61 (100%)
	High	27 (62.8%)	16 (37.2%)	43 (100%)

Table 3. The relationship between lipid profiles and hypertension.

Variable	Hypertension	
	p	r
Total Cholesterol	0.001	0.317**
Triglyceride	0.020	0.217*
LDL	0.091	0.158
HDL	0.423	0.075

DISCUSSION

This study revealed valuable findings regarding the impact of hypertension on total cholesterol and triglyceride levels in middle-aged men, although different patterns were observed for LDL and HDL levels. The Spearman's rank correlation coefficient exhibited a statistically significant positive correlation between total cholesterol and hypertension. This finding suggests that elevated total cholesterol levels are linked to an increased prevalence of hypertension. Triglycerides also showed a positive correlation with hypertension, albeit weaker, and a statistically significant p-value. Triglycerides, which are primarily stored in adipose tissue, have a role in the thickening of blood, resulting in reduced blood flow, elevated blood pressure, and increased cardiac strain. Studies have underscored the association between triglycerides and arterial stiffness, which leads to the narrowing of blood vessel diameter and elevated blood pressure (Suci 2019, Huldani et al. 2020).

In contrast to the findings on total cholesterol and triglycerides, this study found no significant relationship between LDL levels and hypertension. This result aligns with the study conducted by Donni et al. (2023), who revealed that the most frequent LDL levels in hypertensive patients fell within the normal category (47.06%). Patients diagnosed with stage I and II hypertension consistently presented desirable LDL levels, explaining the absence of a significant correlation between LDL and hypertension. Notably, a study examining lipid profiles found that the most prevalent condition was low HDL levels (29.9%). This was followed by high triglyceride levels (20.7%), high total cholesterol levels (14%), and high LDL levels (7.9%) (Wu et al. 2022). These findings highlight the minimal impact of LDL on hypertension.

Consistent with the LDL test results, there was no significant correlation between HDL levels and hypertension observed in this study. The majority of patients had HDL levels that were categorized as borderline high. HDL provides beneficial effects, such as transporting LDL cholesterol away from the blood vessel endothelium and promoting vasodilation by increasing nitric oxide (NO) production (Kuang et al. 2018, Rafsanjani et al.

2019). These findings offer critical insights into the interplay between lipid profiles and hypertension in middle-aged men, aiding in understanding the multifaceted relationship between these variables.

The findings of this study were in line with a number of previous studies. Islam et al. (2014) conducted a study involving 234 patients at the National Centre for Rheumatic Fever and Heart Disease in Dhaka, Bangladesh. The study reported a strong correlation between total cholesterol levels and hypertension (OR=1.1, 95% CI=0.91–1.77, p<0.002). Similarly, a recent study by Chen & Cheng (2022) investigated the relationship between lipid profiles and hypertension in adult Chinese men. They found a robust association between high total cholesterol levels and the occurrence of hypertension among the research subjects. In a study conducted in Indonesia, Feryadi et al. (2014) reported a correlation between total cholesterol levels and hypertension among individuals aged 35–65 from the Minangkabau ethnic group with a significant Chi-square test result (p=0.04).

The research conducted by Osuji et al. (2012) corroborated the findings of this study. In the study, they found that serum triglyceride levels were significantly and positively associated with both systolic and diastolic blood pressure (p<0.001, r=0.063). A complementary study by Kurtkulagi et al. (2022) also demonstrated a significant relationship between triglycerides and hypertension, as supported by the statistical test results (p<0.001). In contrast, several studies found no association between lipid profiles and hypertension. A study conducted by Rahminda et al. (2019) did not observe any association between triglycerides and hypertension among ischemic stroke patients (p=0.27). Furthermore, another study conducted at Budi Asih General Hospital, Jakarta, Indonesia, found similar finding. The study revealed that LDL and hypertension are not correlated. Discrepancies in these findings may be attributed to various factors, such as genetic predisposition, lifestyle patterns, medical history, psychosocial factors, and activities (Kamajaya et al. 2016, Sari 2017).

A study conducted at UKI General Hospital, Jakarta, Indonesia, similarly revealed that the majority of hypertensive patients exhibited normal LDL levels (57.4%). There was no significant relationship observed between LDL and hypertension (Siagian 2017). Additionally, the study conducted by Feryadi et al. (2014) found no significant relationship between LDL and hypertension (p=0.1). Conversely, compared to the aforementioned studies, a significant relationship was observed between LDL levels and hypertension in separate research. The research, however, focused on examining adult women aged 30–50 years. Therefore, the observed differences could be attributed to variations in gender and age within the study samples (Mahmuda et al. 2018).

Previous studies have indicated a lack of a statistically significant relationship between HDL levels and blood pressure. The statistical analysis results of p=0.572 and p=0.268, as reported by Sari (2017) and Putri et al. (2021), respectively, provide evidence of the findings. The findings shown in this study were in opposition to the observations made by Rafsanjani et al. (2019), who observed a relationship between HDL levels and hypertension. Additionally, another study provided support for this contrasting observation. According to Otsuka et al. (2016), low HDL levels were associated with an increased risk of hypertension in individuals with impaired fasting glucose or diabetes. There were several factors that might contribute to the lack of statistical significance in the findings of this study. These factors include alcohol consumption, diabetes, daily food intake, and body mass index (BMI), which were not analyzed in this study.

Hypercholesterolemia contributes to the risk of hypertension through the formation of atherosclerosis-induced foam cells within blood vessels. This process ultimately leads to endothelial dysfunction, arterial wall remodeling, reduced lumen diameter, elevated blood pressure, and organ vasoconstriction (Benslaiman et al. 2022). The pattern that leads to hypertension was evident in this study with the high prevalence of the disease among middle-aged patients, affecting 60–80% of individuals. Notably, the majority of hypertensive patients in this study belonged to the 56–60 age group (48.7%). These results are consistent with age-related pathophysiological changes associated with hypertension, including cholesterol accumulation-induced arterial stiffness, elastin fiber degradation, cross-linking, collagen accumulation, fibrosis, inflammation, smooth muscle cell necrosis, calcification, and the transportation of macro-molecules within arterial walls (Laurent & Boutouyrie 2020, Umar & Mariana 2021). Furthermore, there are several factors that contribute to elevated triglyceride levels in hypertensive patients, such as reduced physical activity due to aging. This can lead to fat accumulation and exercise deprivation (Sondakh et al. 2013, Irnameria et al. 2019).

Notably, six out of eleven hypertensive male patients in this study were identified as smokers, which could further worsen their condition. The potential of carbon monoxide in cigarette smoke to replace oxygen in the bloodstream may contribute to the development of atherosclerosis. The results of a study by Diana et al. (2018) support this association. In their study, 94% of 112 male hypertensive

individuals aged 45–59 who were smokers demonstrated a statistically significant increase in hypertension (p=0.039). However, this study revealed that LDL does not have a significant impact on the progression of hypertension. LDL cholesterol and oxidized LDL (ox-LDL) have been known to enhance local oxidative stress. This can lead to endothelial dysfunction, the release of inflammatory mediators, monocyte macrophage infiltration, foam cell formation, and the activation of angiotensin-converting enzyme (ACE). Ultimately, these processes raise angiotensin II concentration and subsequently increase blood pressure. Ox-LDL can impair endothelial function and vasodilation, resulting in increased aldosterone production, arterial and smooth muscle contraction, and peripheral blood vessel resistance. These pathways play a crucial role in hypertension development and recurrence, with an emphasis on the impact of serum cholesterol, particularly LDL (Wu et al. 2022).

It is noteworthy that a study conducted by Jafar et al. (2020) revealed a consistent pattern of lower HDL levels in men compared to women from adolescence to old age. However, the study found that individuals with hypertension in certain age groups, namely 50–59, 60–69, 80–89, and >90, exhibited higher HDL levels in comparison to those under the age of 50. Moreover, Wu et al. (2022) found that hypertensive men in the age groups of 70–79 and 80–89 years had higher HDL levels compared to individuals aged 50–59. This observation suggests that HDL levels in men tend to increase as they get older. HDL plays a notable role in the development of atherosclerosis, particularly in postmenopausal women. This is due to the protective effects of estrogen, which is effective in safeguarding women during premenopause. The risk of atherosclerosis in women increases with the reduction of estrogen during menopause (Zhao et al. 2018, Sawitri & Maulina 2022). The decrease in estradiol, which is a potent antioxidant, can lead to increased lipid peroxidation and the formation of reactive oxygen species. Consequently, this can affect the composition of pro-inflammatory proteins contained in HDL (Wang et al. 2021). The sample in this study specifically consisted of male hypertensive patients aged 45–60 years. Therefore, these factors might explain the prevalence of HDL levels within the normal range for both stage I and stage II hypertension in this study.

Strength and limitations

This research offers valuable insights into the relationship between lipid profiles and hypertension, especially in male patients aged 45–60. It underscores the significance of adopting a healthy lifestyle, including dietary choices and regular exercise, to help manage lipid profiles and mitigate the risk of hypertension. Such measures are crucial, as hypertension can lead to various non-communicable diseases and increased mortality. However, it is important to acknowledge the limitations of this study. The exclusive focus on hypertensive patients at the Department of Internal Medicine and Department of Cardiology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, indicates that the findings may not be fully representative of the diverse population in East Java. Moreover, the research concentrated solely on hypertension and lipid profiles without considering potential confounding factors, such as comorbidities, BMI, and smoking habits, which could have influenced the outcomes.

CONCLUSION

This study unveiled a notable correlation between hypertension and both total cholesterol and triglyceride levels by considering sex- and gender-specific distinctions. This correlation is particularly noteworthy due to the dearth of research involving middle-aged men in Surabaya, Indonesia. However, this study did not establish any significant correlation between hypertension and low-density lipoprotein (LDL) or high-density lipoprotein (HDL) levels. Hence, a prospective cohort study with a larger sample is required to investigate the potential relationship on a broader scale.

Acknowledgment

The authors would like to express their gratitude to the Department of Internal Medicine and Department of Cardiology at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, for providing access to their facilities.

Conflict of interest

None.

Ethical consideration

This study was approved by the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, with reference No. 1062/LOE/301.4.2/X/2022 dated 6/10/2022.

Funding disclosure

None.

Author contribution

AN contributed to the conceptualization and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, and collection and assembly of

the data. AT, UM, S, and IMSH contributed to the conceptualization and design, analysis and interpretation of the data, critical revision of the article for important intellectual content, final approval of the article, and provision of study materials, as well as administrative, technical, and logistic support.

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Original Research Report

EFFECT OF SHORT-TERM EXTRA MALTODEXTRIN DURING A DIET BREAK ON THE RESISTANCE TRAINING PERFORMANCE OF *Rattus norvegicus*

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ABSTRACT

Poor diet with a purpose to lose a certain body weight or body fat can impair muscle protein synthesis. This results in muscle loss and poor performance in physical training, particularly resistance training. This study aimed to determine the short-term effect of extra carbohydrates, specifically in the form of maltodextrin, during a diet break on the resistance training performanced by rats. This study was an experimental laboratory study with a randomized posttest-only control group design. Twenty-seven male rats, aged 3 months with a weight range of 140–165 g, were randomly and equally assigned into three groups: KN (standard diet), KP (75% calorie intake), and K1 (65% calorie intake with a diet break and extra maltodextrin every week). These diets were administered for four weeks, during which the rats had *ad libitum* feeding. Additionally, the rats underwent ladder-climbing training three times a week. The body weight was measured pre- and post-treatment, while the performance in resistance training was evaluated post-intervention using a ladder climbing platform. There was no significant difference in the weight before and after treatment, with $p>0.05$ for the increments (Δ) among KN (14.00±9.89 g), KP (13±9.5 g), and K1 (20.89±14.77 g). According to the posttest assessment results, only 17 out of 27 rats succeeded in the maximum weightlifting test. This study showed that a short-term high-carbohydrate diet break does not improve the resistance training performance of rats. Further research is necessary to ascertain the outcomes of the treatment implemented over an extended period of time.

Keywords: Diet break; carbohydrates; ladder-climbing exercise; calorie restriction; human and health

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Article history
●Submitted 10/9/2023 ● Revised 23/10/2023 ● Accepted 7/11/2023 ● Published 10/9/2023

How to cite: Indiarto MI, Irwadi I, Lukitasari L, et al (2023). Effect of Short-Term Extra Maltodextrin during a Diet Break on the Resistance Training Performance of *Rattus norvegicus*. Folia Medica Indonesiana 59 (3), 329-334, https://doi.org/10.20473/fmi.v59i4.39677

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

Highlights:
1. The ongoing advancement of dietary research has significant importance in the exploration of strategies to optimize the impact of nutrition on performance during resistance training.
2. This study provides a prompt for future research to explore the effectiveness of incorporating an additional dietary strategy that may enhance resistance training performance.

INTRODUCTION

According to the most recent guidelines of the World Health Organization and the American College of Sports Medicine, regular resistance training can provide comprehensive health benefits. These advantages include a reduction in mortality associated with cardiovascular disease, hypertension incidence, type 2 diabetes, various forms of cancer, and adiposity. Additionally, regular resistance training has been found to improve mental health by reducing depressive and anxiety symptoms (Pranoto et al. 2023). The goal of maintaining muscle size and strength during a prolonged period of energy restriction is to achieve a leaner physical profile. In individuals who do not use anabolic agents, it is exceedingly challenging to maintain or even progress a load due to a lack of energy during the prolonged period of energy restriction (Anstey et al. 2018).

The ketogenic dietary regimen was initially used as

an alternative to medication for people with epilepsy. However, it has now become a widely utilized therapeutic approach for patients with obesity, diabetes mellitus, and cancer. According to current cancer research, it has been observed that the addition of tumor necrosis factor (TNF) and interleukin-6 (IL-6) inhibitors to high-fat diets can reduce inflammation and mucosal damage in the colons of mice. Consequently, this preventive measure hinders the growth of tumors (Kern et al. 2018). The aforementioned dietary regimen has evolved and become a method that is incorporated into everyday life. The typical macronutrient distribution for a ketogenic diet consists of 75% fat, 20% protein, and 5% carbohydrate (Laksana et al. 2021).

The combination of chronic energy restriction and low-carbohydrate intake has been observed to impair anaerobic exercise performance. This may lead to muscle atrophy and a decrease in the number of calories burned during exercise, ultimately resulting in a reduction in the total daily energy expenditure (TDEE). A decrease in TDEE might lead to a stagnation in the pace of weight loss. If individuals are unable to make practical adjustments, their weight loss progress may stall. This can cause a decrease in motivation that ultimately results in failure to achieve weight loss goals (Hall 2018). Recent research has investigated intermittent energy restriction as a potential strategy to mitigate the negative effects associated with prolonged and extreme energy restriction. Intermittent energy restriction has been demonstrated to be superior compared to continuous energy restriction in terms of weight reduction efficiency, metabolic adaptation, conserved lean body mass, and training performance (Byrne et al. 2018).

The implementation of a carbohydrate-rich diet in the short term has the potential to improve performance during training sessions by increasing stored muscle glycogen and delaying the onset of fatigue. However, until recently, most diet break or intermittent energy restriction research primarily focused on body composition, compensatory metabolic response, and related indicators such as insulin (Henselmans et al. 2022, Kim et al. 2022). There is a dearth of studies on the impact of a diet break or intermittent energy restriction on exercise performance, specifically in the context of resistance training. Consequently, there is a pressing need for further study in this area. This concept pertains to those who adhere to calorie restriction, such as athletes who are preparing for competitive weight requirements or obese patients undergoing strict energy restriction. The foregoing background outlines the objective of this study, which was to examine the effect of a high-carbohydrate diet on the performance of calorie-restricted *Rattus norvegicus* during resistance training.

MATERIALS AND METHODS

This study was experimental laboratory research with a randomized posttest-only control group design. Wistar strain rats (*Rattus norvegicus*) were used as animal models due to their anatomical and physiological similarities to humans (Santana et al. 2019). This study used 27 male rats aged 3 months within the weight range of 140–165 g. During the four-week treatment, the rats were randomly divided into three groups consisting of nine rats each: KN, KP, and K1. The KN group was provided with a standard diet equivalent to 5 grams per 100 grams of body weight. The KP group was given 75% of the standard diet, while the K1 group received 65% of the standard diet. However, the diet of the K1 group included a two-day break and extra maltodextrin intake (2.25 g) every week until equal calorie consumption with the KP group was achieved. The animal models were acclimatized for one week, during which they were provided with *ad libitum* feeding and were familiarized with ladder-climbing exercise. The research was conducted for five weeks in the Biochemistry Laboratory for Experimental Animals, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

The bodyweight of the rats was measured pre- and post-intervention using a digital scale (Harnic HL-3650 Heles, China), with a maximum capacity of 5 kg and a graduation of 1 g. The ladder-climbing training equipment was constructed using wooden materials. The dimensions of the equipment were measured to be 110 cm in height and 18 cm in width. There was a 2 cm gap between each staircase, and the equipment had an inclination angle of 80 degrees. Located at the top of the ladder, a shelter in the form of a box with dimensions of 20 x 20 x 20 cm was provided as a resting area for the exercising rats (Figure 1). The maximum weightlifting test was conducted post-treatment. The rats climbed the ladder while carrying a load that was equivalent to 75% of their own weight. This procedure was repeated until the animals failed to climb, hence obtaining the maximum capacity in the weightlifting training. The maximum capacity was established after the rats demonstrated consistent failure to climb the ladder for three consecutive attempts. The resistance training was performed twice per week, utilizing loads that were reduced to 50% of those used in the previous weightlifting test. Each resistance training session consisted of three sets and three repetitions. This exercise conformed to the recommendations offered by the American Heart Association, which advocate for two sessions of resistance training per week. Notably, the training

put more emphasis on strengthening the lower body muscles than the upper body muscles, given their essential function in carrying out everyday tasks (Fauzi et al. 2022).

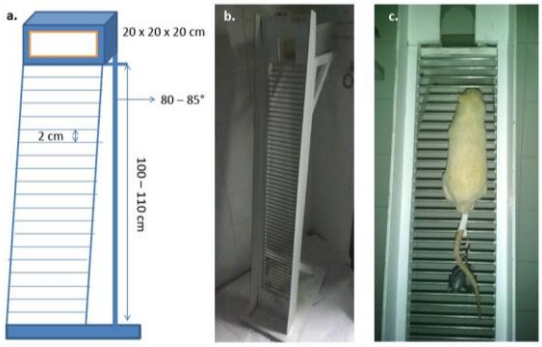

Figure 1. Vertical ladder-climbing platform for resistance training (Neto et al. 2016).

In accordance with the study carried out by Petrie & Watson (2013), the data analysis in this study was performed with the assistance of IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA). The data were presented in numerical values and percentages. Numerical data were presented as mean (standard deviation) and standard error if a normal distribution was present. The posttest data for Groups KN, KP, and K1 were analyzed using the Chi-square test. However, it was found that the necessary criteria for this test were not met. As a result, a follow-up statistical analysis method, the Fisher's exact test, was employed to examine the differences between the groups. The Shapiro-Wilk test was employed to determine normality. The mean difference for a normal distribution was determined using an independent t-test, while the Mann-Whitney test was employed for a skewed distribution. The statistical significance was set at $p<0.05$.

RESULTS

A total of twenty-seven male rats of the Wistar strain, with an overall age of 3 months and a weight range of 140–165 g, were distributed into three distinct groups: KN, KP, and K1. The bodyweight measurements pre- and post-intervention exhibited a normal distribution ($p>0.05$).

Since there is a connection between bodyweight and strength, a bodyweight analysis was performed between groups, which revealed normally distributed differences ($p>0.05$). Furthermore, a Mann-Whitney comparative test was performed. The characteristics of the body weight of subjects are reported in Table 1.

Table 1. The rats' weight post-intervention according to the ANOVA results.

Groups	Mean±SD	n	p (Fisher's exact test)
KN	182.33±23.61	9	0.875
KP	181.56±24.23	9	
K1	177.44 ±15.46	9	

Note: ANOVA=Analysis of variance.

The statistical analysis of the maximum weightlifting test resulted in $p=0.069$. This value indicated that there was no significant relationship between the difference in body weight and ladder-climbing performance in all groups, regardless of the presence or absence of calorie restriction. The results shown in Table 2 demonstrate that there is no statistically significant difference between rats that succeeded in the maximum weightlifting test and those that did not.

Table 2. Results of the post-intervention maximum weightlifting test.

Groups	Success	Failure	Total
KN	3 (33.3%)	6 (66.7%)	9
KP	8 (88.9%)	1 (11.1%)	9
K1	6 (66.7%)	3 (33.3%)	9
Total	17 (63.0%)	10 (37.0%)	27
p (Fisher's exact test)	0.069		

DISCUSSION

In this study, the rat models were distributed into three groups, consisting of one control group and two treatment groups. The first treatment group was subjected to a calorie restriction, whereas the second treatment group underwent the same regimen but included a diet break and extra carbohydrate intake. The treatment was implemented using a food-rationing procedure tailored to each group. This dietary treatment aligns with research conducted on human subjects (Campbell et al. 2020). The variables assessed in response to the intervention were the results of the maximum weight lifting test. The assessment was performed to evaluate the muscular strength of the rats engaged in resistance training, specifically ladder climbing.

The observed pattern of body weight changes among the rats in this study showed a noticeable weight increase, albeit without a significant difference. This finding demonstrates that an increase in body weight does not consistently affect performance during ladder-climbing resistance training. Individuals classified as obese may have higher muscle mass but lower muscle quality compared to those with a normal weight (Cava et al. 2017). The observed weight gain in the rats, while being subjected to a 25% reduction in food intake, contradicts prior

research findings on the effects of food restriction on body weight. This research analyzed the effect of extending the duration of calorie restriction by introducing maltodextrin supplementation to a specific group, which may potentially produce variations in athletic performance during ladder-climbing resistance training. It is noteworthy that the rats in this study had never received any form of resistance training intervention, hence allowing for the initiation of early muscle growth.

According to prior research, it has been demonstrated that an increase in muscle strength may become evident within three weeks of resistance training. Hypertrophy has been identified as an important factor that contributes to the increase in muscle strength (Loenneke et al. 2019). The muscles that have a role in hypertrophy are the flexor hallucis longus, soleus, extensor digitorum longus, and triceps brachialis. Additionally, several studies have reported a growth in the size of the gastrocnemius and flexor digitorum longus muscles (Neto et al. 2013, Cassilhas et al. 2013). In a study with an 8-week treatment, the flexor hallucis longus muscle was dissected and weighed after the completion of ladder climbing training. The measurement of the wet weight showed a 23% increase compared to both the non-trained and control groups (Cassilhas et al. 2013). According to these findings, it may be inferred that the observed weight gain in the rats might be attributed to early-phase hypertrophy. A study conducted in the field of bodybuilding found that adult males can gain up to 7.2 kg of lean mass within the first year of training (Benito et al. 2020).

This study demonstrated muscle gain or hypertrophy in rats, even when in a calorie deficit. This finding supported a study conducted by Barakat et al. (2020) who found the occurrence of hypertrophy in non-trained individuals. During a calorie-restricted diet period, skeletal muscle hypertrophy may manifest in individuals belonging to several categories: individuals who are untrained and initiating resistance training, obese individuals, detrained athletes, or those who quit resistance training and eventually start out again. The prerequisites for this occurrence are nitrogen balance or a surplus through increased consumption of dietary protein, a well-structured resistance training plan, and a calorie intake that is equivalent to or slightly below the TDEE (Krzysztofik et al. 2019). A full comprehension of this subject matter requires further research that includes a thorough examination of the gastrocnemius, flexor hallucis longus, soleus, and flexor digitorum longus muscles. This will enable a comprehensive analysis of the differences between the groups under study.

During the treatment period of this study, the rats were provided with feed in accordance with the predetermined quantity for each group. Both the KP and K1 groups received the same weekly calorie intake (75% of the KN group's standard diet), but with different timing and food sources. The results of this study showed that weight gain was observed in all groups following the treatment. Surprisingly, the KP group demonstrated superior performance in the post-treatment maximum weight lifting test. The KP group involved the heaviest rat, weighing 227 g, despite the similarity in average weight across all groups. However, the phenomenon counters our hypothesis, which predicted that the KP group might exhibit the poorest performance in the post-treatment maximum weight lifting test, while the KN group could be the highest performing group due to the absence of calorie restriction. Compared to the KP group, the K1 group supposedly exhibited superior performance as a result of increased glucose intake, albeit with the same net weekly calories. There was evidence suggesting that the calorie restriction encouraged the rodents to nibble on the bedding material composed of rice husk, as observed in this experimental study. The nutritional value of rice husk is comparatively suboptimal when compared to BR-1 feed, which is composed of 3.1% protein, 2.7% fat, 12.5% water, and 17.5% ash (Partama et al. 2019). Furthermore, a low-protein diet has been shown to potentially have an adverse effect on the composition and function of gut bacteria. Wu et al. (2022) found that mice subjected to a low-protein diet had a decreased abundance of cecal bacteria, specifically *Alistipes* sp., *Roseburia* sp., and *Muribaculaceae*. This composition is assumed to be ideal for promoting muscle hypertrophy, hence making the post-treatment maximum weightlifting test suboptimal.

Strength and limitations

The publication of this study holds significance as it enables interested researchers to further investigate this subject matter with an improved experimental design, an extended time frame, and the inclusion of additional variables to yield more robust outcomes. One of the limitations of this study pertained to the absence of a maximum weightlifting test before the administration of the treatment. Future studies should carefully consider the aforementioned factors and develop a refined research design according to the findings presented in this paper.

CONCLUSION

A short-term weekly diet break with additional carbohydrate intake in the form of maltodextrin has no significant difference in the performance of resistance training, despite the presence or absence of calorie restriction.

Acknowledgment

The authors would like to express their gratitude to Universitas Airlangga, Surabaya, Indonesia, for facilitating the execution of this experimental study.

Conflict of interest

None.

Ethical consideration

This study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, with reference No. 68/EC/KEPK/FKUA/2022 on 31/3/2022.

Funding disclosure

None.

Author contribution

MII contributed to the conceptualization and design, analysis and interpretation of the data, drafting of the article, final approval of the article, provision of study materials, and collection and assembly of the data. II contributed to the collection and assembly of data, critical revision of the article for important intellectual content, and final approval of the article. LL contributed to the conceptualization and design, collection and assembly of data, critical revision of the article for important intellectual content, and final approval of the article. A contributed to the analysis and interpretation of the data, the drafting of the article, a critical revision of the article for important intellectual content, and the final approval of the article.

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Original Research Report

LYMPHOCYTE COUNT AND SARS-CoV-2 ANTIBODY LEVEL IN HEALTHY DONORS' BLOOD AT AN INDONESIAN BLOOD TRANSFUSION CENTER

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a virus that infects the respiratory system by attacking the mucous and epithelial cells. This infection commonly leads to an increase in lymphocyte count as an immune response to invading pathogens. Moreover, antibodies bind and inactivate foreign substances to destroy pathogens and inhibit their replication. These mechanisms prompt the objective of this study, which was to define the relationship between lymphocyte count and SARS-CoV-2 antibody level. This analytical observational study used a cross-sectional approach with quantitative analysis methods and purposive sampling. Healthy donors who had received coronavirus disease (COVID-19) vaccines provided the samples for this study. A total of 30 blood samples were collected from the Blood Transfusion Center of the Indonesian Red Cross Surabaya Area. This study was conducted in May 2022 at two distinct locations. The examination of lymphocytes was carried out using the flow cytometry method in the Hematology Laboratory, Department of Medical Laboratory Technology, Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia. In addition, the antibody titer test using the enzyme-linked immunosorbent assay (ELISA) method was performed in the Immunoserology Laboratory of the Surabaya Health Laboratory Center, Surabaya, Indonesia. The analysis revealed an average lymphocyte concentration of 2.2633 x 10^3/µl and an average antibody level of 0.2197 according to the optical density (OD) ratio. The data analysis was performed using Spearman's rank correlation statistical test ($p<0.005$), and the results indicated a lack of significance with $p=0.262$. In conclusion, there is no relationship between total lymphocyte count and SARS-CoV-2 antibody level.

Keywords: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); virus; antibody; lymphocyte; enzyme-linked immunosorbent assay (ELISA)

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Article history
●Submitted 12/09/2022 ● Revised 7/10/2023 ● Accepted 22/11/2023 ● Published 10/12/2023

How to cite: Prasasti AG, Woelansari ED, Suhariyadi, et al (2023). Lymphocyte Count and SARS-CoV-2 Antibody Level in Healthy Donors' Blood at an Indonesian Blood Transfusion Center. Folia Medica Indonesiana 59 (4), 335-341, https://doi.org/10.20473/fmi.v59i4.38954

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

Highlights:
1. It is essential to conduct research on SARS-CoV-2 for the purpose of acquiring further understanding, especially concerning the production of antibodies examined using antibody titer blood tests.
2. Although the relationship between the examined variables is not significant, this study offers valuable information on blood test results after the COVID-19 vaccination, which can serve as scientific evidence for further research.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a group of ribonucleic acid (RNA) viruses that originate from animals and can be transferred to humans. The virus can penetrate the body by binding to the receptor angiotensin-converting enzyme 2 (ACE2), which is identifiable by toll-like receptor 3 (TLR3). The toll-like receptor (TLR) activates the natural immune response, which in turn initiates the formation of an adaptive immune response. This includes the stimulation of B cells to differentiate into plasma cells and switch the isotypes of the antibodies produced. TLR3 plays a role in the presentation of viral antigens by dendritic cells to the cluster of differentiation 8^+ ($CD8^+$) cytotoxic T lymphocytes. It also influences the modulation and regulation of T lymphocyte tolerance. In the adaptive immune system, antibodies can function by binding to viral particles and blocking the infection of host cells. T cells have a significant role in recognizing and destroying

virus-infected cells. A history of prior viral infections can increase the ability of effector cells due to the development of immunological memory, which facilitates the activation of recall responses (Farber et al. 2016). However, several studies have demonstrated that the immune response elicited during an infection appears uncontrolled. The hyperactivation of monocytes and macrophages can lead to increased neutrophil activity, upregulation of interleukin 6 (IL-6) expression, elevated levels of C-reactive protein (CRP), and a reduction in lymphocyte count (Laili 2021).

In SARS-CoV-2 infection, lymphocytes function as antigen-presenting cells (APCs) and effectors capable of producing chemokines and cytokines. Lymphocytes can differentiate and proliferate into T helper cells, cytotoxic T cells, or B cells, depending on the stimuli they receive. B lymphocytes can produce two types of antibodies: immunoglobulin M (IgM) that appears during the acute phase of an infection and immunoglobulin-G (IgG) that indicates a persistent infection. IgM antibodies can be identified in the blood serum of individuals who have been infected with SARS-CoV-2 between days 3 and 6 after the infection. On the other hand, IgG can be identified eight days after the onset of symptoms. Seroconversion typically occurs within the second week following the onset of symptoms. Previous studies have suggested that patients who have recovered from SARS-CoV-2 infection can develop long-lasting immunity, characterized by high levels of IgG in the antibody titer. Antibodies present in plasma can be found at reasonably stable levels for at least 5 to 8 months post-infection (Röltgen & Boyd 2021).

Previous studies have demonstrated that lymphocyte counts can serve as an indicator to monitor the treatment and diagnosis of patients with infections. In addition, a decrease in lymphocyte count is the main characteristic for assessing the severity of the disease (Shereen et al. 2020, Lagunas-Rangel 2020). Given the available information, we performed antibody titers to investigate the relationship between total lymphocyte count and antibody level in healthy donors who might have developed antibodies against SARS-CoV-2.

MATERIALS AND METHODS

This study used an analytical observational design with a cross-sectional approach and quantitative analysis methods. Antibody titers were performed to determine the relationship between lymphocytes and SARS-CoV-2 antibodies. The study population consisted of healthy individuals who were donors at the Blood Transfusion Center of the Indonesian Red Cross Surabaya Area. The participants were selected through purposive sampling based on specific criteria, which included healthy individuals who had passed a series of screenings and met the eligibility requirements for blood donation between March and April 2022 (Kesmodel 2018). They also had to be declared healthy and either native citizens or residents of Surabaya, Indonesia. This study excluded individuals who were deemed unqualified as blood donors, ineligible for blood donation procedures, or who came from or resided outside the city of Surabaya (Zetterstrom & Waernbaum 2022). The participants provided blood samples in 3 mL EDTA tubes for lymphocyte counts and in 3 mL plain tubes for antibody titers. Furthermore, the blood donors who had developed antibodies against SARS-CoV-2 were screened using a qualitative antibody test. This study received ethical approval from the Health Research Ethics Committee of Poltekkes Kemenkes Surabaya, Surabaya, Indonesia, with reference No. EA/841/KEPK-Poltekkes_Sby/V/2022 on 23/3/2022.

The data obtained consisted of primary data with a ratio scale derived from examination results. The blood specimen examinations included antibody titers and lymphocyte counts carried out at two different locations. The flow cytometry method was used to examine the lymphocyte count (Normal values: 18 – 42 x 10 3 /µl) at the Hematology Laboratory, Department of Medical Laboratory Technology, Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia. Additionally, the enzyme-linked immunosorbent assay (ELISA) method was used in the antibody titer test performed in the Immunoserology Laboratory of the Surabaya Health Laboratory Center, Surabaya, Indonesia. The antibody titer data were assessed by examining the optical density Results higher than the cutoff values of 0.300 optical density were reported as positive for the anti-SARS-CoV-2 antibodies (Clark & Engvall 2018).

Once a ratio scale was derived from the data, the Kolmogorov-Smirnov test was conducted to assess the normality of the data distribution. IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) was utilized in the data analysis (Hinton et al. 2014). Following the confirmation of the normal distribution of the data, the investigation proceeded with the Pearson's correlation test. On the other hand, the non-normally distributed data were assessed using the Spearman's rank correlation test (Sedgwick 2014).

RESULTS

This research was carried out by examining total lymphocyte counts and antibody titers using EDTA tubes and plain tubes with a blood sample volume of

3 mL for each type of tube. Table 1 presents the results of the qualitative antibody test conducted on the blood samples to detect their SARS-CoV-2 antibodies.

Table 1. Results of the qualitative antibody screening test of the blood samples.

Sample codes	Examination results	
	IgG	IgM
X070401	Reactive	Non-reactive
X070402	Reactive	Non-reactive
X070403	Reactive	Non-reactive
X070405	Reactive	Non-reactive
X070406	Reactive	Non-reactive
X041101	Reactive	Non-reactive
X041102	Reactive	Non-reactive
X041103	Reactive	Non-reactive
X041104	Reactive	Non-reactive
X041105	Reactive	Non-reactive
X041106	Reactive	Non-reactive
X041107	Reactive	Non-reactive
X041108	Reactive	Non-reactive
X041109	Reactive	Non-reactive
X041110	Reactive	Non-reactive
X041301	Reactive	Non-reactive
X041302	Reactive	Non-reactive
X041303	Reactive	Non-reactive
X041304	Reactive	Non-reactive
X041305	Reactive	Non-reactive
X041306	Reactive	Non-reactive
X041307	Reactive	Non-reactive
X041308	Reactive	Non-reactive
X041309	Reactive	Non-reactive
X041310	Reactive	Non-reactive
X041311	Reactive	Non-reactive
X041312	Reactive	Non-reactive
X041313	Reactive	Non-reactive
X041314	Reactive	Non-reactive
X041315	Reactive	Non-reactive

The specimens used in this study passed the initial screening that was performed using a qualitative antibody test. The test revealed positive results, which indicated that all specimens had an IgG antibody response. In comparison, the specimens did not exhibit a positive IgM antibody response. This might be related to the fact that one of the donor requirements was to have a minimum interval of 3–6 months after the SARS-CoV-2 infection and vaccination.

The results showed that the average value of the total lymphocyte counts in the blood samples collected from healthy donors was within the normal range. The data indicated that the blood donors had favorable levels of physical activity and medication usage. Most of the participants in this study had lymphocytes that fell within the normal range (Table 2). It was because the participants were in good health and were not suffering from or being exposed to infectious diseases.

Tabel 2. Lymphocyte counts and results of the SARS-CoV-2 antibody test.

Sample codes	Lymphocytes (x 10^3 /µl)	Optical density
X070401	2.3	0.2508
X070402	4.3	0.2184
X070403	2.7	0.2667
X070405	3.8	0.1168
X070406	2.3	0.2168
X041101	1.2	0.769
X041102	1.9	0.16
X041103	1.8	0.0866
X041104	1.6	0.1403
X041105	1.6	0.1918
X041106	1.4	0.183
X041107	2.1	0.2012
X041108	1.5	0.2436
X041109	1.9	0.1637
X041110	1.5	0.3373
X041301	1.5	0.3028
X041302	1.9	0.1777
X041303	1.7	0.366
X041304	2.2	0.2142
X041305	2.6	0.1984
X041306	2.7	0.1351
X041307	2.5	0.2563
X041308	2.6	0.292
X041309	3.7	0.3075
X041310	2.2	0.1348
X041311	2.8	0.1066
X041312	2.5	0.1861
X041313	2.3	0.1519
X041314	2.0	0.1091
X041315	2.8	0.1054

Tabel 3. Results of the Spearman's correlation test of lymphocyte count and antibody level.

	Lymphocyte count	Antibody level
Correlation coeff.	-0.211	-0.211
Significance	0.262	0.262

Table 3 shows that there is no significant relationship between lymphocyte count and antibody level variables. The results of the Spearman test on the relationship between lymphocyte count and SARS-CoV-2 antibody level revealed a coefficient of 0.211 with a negative correlation. This indicated that as the antibody level increased, it was followed by a decrease in lymphocyte count.

DISCUSSION

Lymphocytes are a subset of agranulocyte leukocytes, which have various important functions in the immune system, specifically in responding to attacks from microorganisms, foreign macromolecules, and cancer cells. None of the participants in this study exhibited decreased lymphocyte levels, which refers to a level of lymphocytes falling below the normal range due to the pre-existing lymphocytes in the tissue. The decrease in lymphocyte count can be caused by the migration of lymphocytes from the bloodstream to the surrounding tissues (Nourshargh & Alon 2014). The maximum load also causes a reduction in antibody production and a general decrease in lymphocyte function. In this study, it was found that only a single individual (3.33%) exhibited a lymphocyte count above the normal range. The total lymphocyte count might increase due to lymphatic leukemia, mononuclear infection, or viral infection. Elevated levels of lymphocytes may arise in the presence of cellular damage in tissues or organs, necessitating a response to the elimination of the damaged or dead cells (Nisnawati et al. 2021).

During the humoral immune response to SARS-CoV-2, the cluster of differentiation 4^+ T cells ($CD4^+$ T cells) interact with the B cells. The attachment of antigen to receptors on the surfaces of B cells induces the activation and differentiation of B cells into plasma cells that produce antibodies, specifically IgM and IgG (Cox & Brokstad 2020). IgM and IgG antibodies typically appear during the second week post-virus exposure, followed by the emergence of neutralizing antibodies capable of counteracting viral infection. The production of IgM starts to decline in the fourth week and will cease three weeks later. If an individual continues to produce IgM antibodies for more than one month, it suggests that the SARS-CoV virus is still replicating in the body, which is a symptom of an acute stage of disease (Crawford et al. 2021).

Unlike IgM, IgG can remain in the body for an extended period of time. IgG produced in response to SARS-CoV infection can still be detectable up to 24 weeks later. Previous studies have demonstrated that IgG and neutralizing antibodies may persist for up to two years post-infection, which indicates that IgG possesses protective properties against re-infections. According to the research conducted by Ndzouboukou et al. (2021), the decrease in IgG antibody levels approximately three months after SARS-CoV-2 exposure is a natural occurrence within the life cycle of antibodies. Another study revealed a decline in antibody levels over a period of eight months post-infection. The study further showed that there was a slight decrease in T cell counts, although with considerable variations across individuals. The challenging measurement of B cells revealed that the cell counts remained stable despite occasional increases (Hutapea 2022). This suggests that even though there is a decline in antibodies, several components will have been present at sufficient levels. These components can restart antibody production and coordinate immune responses against viruses. The specific mechanisms that lead to post-infection immunologic memory also serve as a foundation for immunity acquired through vaccination.

The production of antibodies may lead to changes in blood components, particularly those related to proteins and white blood cells. Vaccination has been known to increase blood protein levels, leukocyte counts, and erythrocyte sedimentation rates (Kellam & Barclay 2020). Three to five weeks following vaccination, the average levels of white blood cells and protein in the blood will return to their normal ranges. Blood characteristics will likewise return to their original state. However, variations in physical activity, medication, and infection severity can affect the total lymphocyte count (Tiara et al. 2016). On the other hand, antibodies may not form optimally after vaccination due to several factors, i.e., primary and secondary factors. The primary factors include an inaccurate immunization schedule and a history of infection, while the secondary factors consist of age, sex, nutritional status, immune status, and comorbidities (Nisnawati et al. 2021).

Jackson et al. (2020) conducted a comparative study involving unvaccinated coronavirus disease (COVID-19) survivors and vaccinated healthy individuals. It was found that the COVID-19 survivors developed neutralizing antibodies, albeit not to the same level as the vaccinated individuals. If the COVID-19 survivors had received a vaccination, their levels of effective antibodies would have been higher than those of the healthy individuals. Neutralizing antibodies were found to be significantly lower in those who had not been infected but had received a second dose of vaccine than in survivors who had only received one dose of vaccine (Röltgen & Boyd 2021).

Theoretically, as one gets older, fewer naïve T cells are available to respond to vaccinations. The standard ratio of cluster of differentiation 4 (CD4) to cluster of differentiation 8 (CD8) cells rises tremendously because CD8 T cells significantly decrease with age. Aging causes a decline in the T cell receptor diversity of CD8 and CD4 cells, which subsequently results in decreased T cell survival. A shift in the generation of short-lived effector T cells, rather than memory precursor cells, is one of the qualitative changes that can impair follicular T cell helper responses to vaccination. In old age, B cell

counts remain relatively stable, but fewer functional antibodies are produced due to decreased expression of certain proteins (Cheng et al. 2021).

Women have higher activity levels of cytotoxic T cells and lymphocytes compared to men. This includes the expression of proinflammatory and antiviral genes that are upregulated in T cells. Several non-specific indicators of cell-mediated immunity have been demonstrated to increase in women, suggesting that they experience higher lymphocyte proliferation and immunological sensitivity to foreign substances. Generally speaking, women consistently have higher basal levels of immunoglobulins and antibody responses to vaccinations compared to men (Klein et al. 2015).

The relationship between obesity and the immune response in humans has been documented in prior studies. It has been demonstrated that a higher body mass index (BMI) lowers immune function and antibody levels post-vaccination. Additionally, an association has been established between a larger abdominal circumference and lower antibody levels in COVID-19 patients (Ross et al. 2020, Pratikstha 2021). Increased inflammatory cytokine production has been linked to obesity. These cytokines include interleukins, interferons, and tumor necrosis factor (TNF), which are indicative of low-grade chronic inflammation and can impair both innate and adaptive immune responses. Lower antibody levels in an immunological response to the SARS-CoV-2 vaccine were associated with a higher BMI considered as obesity, according to a study conducted on Italian healthcare workers (Pellini et al. 2021).

COVID-19 patients with comorbidities are not recommended to receive vaccinations unless under the supervision of the treating doctor. Obesity, diabetes, and cardiovascular disease are among the comorbidities. These conditions worsen the clinical outcome of the COVID-19 infection. One of the salient characteristics of SARS-CoV-2 infection is lymphopenia, which is associated with the severity of the disease. It was found that CD4+ and CD8+ T cells, B cells, and killer T cells were all impacted in lymphopenia patients (Choi & Cheong 2021).

In this study, the administration of various vaccine boosters had an impact on the antibodies that developed in the blood of the donors. The levels of antibodies generally decrease six months after the administration of the primary dose. Therefore, additional doses are required to boost individual protection, particularly in vulnerable populations. According to the circular issued by the Ministry of Health of the Republic of Indonesia (2022), there are two mechanisms of administering booster shots: homologous (in which the type of booster is the same as the primary dose) and heterologous (in which the type of booster differs from the primary dose). These varied mechanisms might result in different levels of antibodies being formed in each individual.

Strength and limitations

The samples in this study were gathered in almost identical conditions, thereby reducing research bias. This study illustrated the outcomes of multiple blood tests conducted after COVID-19 vaccinations. As a result, the data in this study can provide a scientific overview of the public health significance of the COVID-19 vaccinations. However, this was a unicentric study that covered a narrow geographic area. Additionally, the variables examined in this study were limited to total lymphocyte count as well as antibody level with an observation of the optical density of blood samples. Since the subjects were less homogeneous, it was challenging to obtain accurate data on the formation of antibodies by considering the duration of vaccine administration.

CONCLUSION

There was no relationship between lymphocyte count and SARS-CoV-2 antibody level in the blood of healthy donors. The lymphocyte counts and antibody levels of the blood donors were within normal limits.

Acknowledgment

We would like to thank the staff of the Blood Transfusion Center of the Indonesian Red Cross Surabaya Area for their hospitality and assistance during the process of gathering data and specimens.

Conflict of interest

None.

Ethical consideration

This study was approved by the Health Research Ethics Committee of Poltekkes Kemenkes Surabaya, Surabaya, Indonesia, with reference No. EA/841/KEPK-Poltekkes_Sby/V/2022 on 23/3/2023.

Funding disclosure

None.

Author contribution

AGP contributed to the conception and design, analysis and interpretation of the data, drafting of the

article, provision of study materials, and administrative, technical, and logistic support. EDW contributed to the conception and design, critical revision of the article for important intellectual content, final approval of the article, and provision of study materials. S contributed to the statistical expertise, acquisition of funding, and collection and assembly of data. ADA contributed to the critical revision of the article for important intellectual content, final approval of the article, and administrative, technical, and logistic support.

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Original Research Report

CHARACTERISTICS OF APHASIA IN ISCHEMIC STROKE PATIENTS AT A NATIONAL BRAIN CENTER IN INDONESIA

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ABSTRACT

Different types of aphasia may occur due to lesions in various brain regions following ischemic strokes. Global aphasia was the most prevalent type of aphasia before the COVID-19 pandemic. However, stroke incidence and mortality rose during the pandemic. This study aimed to identify the types, clinical and radiological features, and management of aphasia in ischemic stroke cases during the pandemic. This study was a descriptive study with a cross-sectional design. The total sampling technique was used for the sampling process. The research samples were ischemic stroke subjects with aphasia who were diagnosed between January 1 and December 31, 2021, at the National Brain Center Prof. Dr. dr. Mahar Mardjono Hospital, Jakarta, Indonesia. The statistical analysis was performed using IBM SPSS Statistics for Mac, Version 25.0 (IBM Corp., Armonk, N.Y., USA). The results of this study showed that 162 aphasic subjects had suffered from ischemic strokes. The age range of the subjects was 34–87 years old. The majority of the subjects were male (59.9%) and aged 55–65 years (37.0%). The three most common risk factors were hypertension (90.1%), diabetes mellitus (50.0%), and dyslipidemia (75.9%). Motor aphasia (33.3%) and global aphasia (43.8%) were the most prevalent types of aphasia among the subjects. The parietal lobe was the main location of the causative lesions, as demonstrated by 38 global aphasic subjects and 47 motor aphasic subjects. The therapies administered to the subjects consisted of speech therapy (85.2%), antiplatelet therapy (98.1%), anticoagulants (19.1%), recombinant tissue plasminogen activator (rTPA) (1.2%), and neuroprotectors (3.0%). This study concluded that global aphasia was the most common type of aphasia among ischemic stroke patients during the pandemic, with the parietal lobe as the primary location of the causative lesions.

Keywords: Aphasia, ischemic stroke, health risk, cardiovascular disease, obesity

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Article history
●Submitted 19/1/2023 ● Revised 12/10/2023 ● Accepted 25/11/2023 ● Published 10/12/2023

How to cite: Danardhono RH, Saleh AY, Theresa RM, et al (2023). Characteristics of Aphasia in Ischemic Stroke Patients at a National Brain Center in Indonesia. Folia Medica Indonesiana 59 (4), 342-351, https://doi.org/10.20473/fmi.v59i4.42746

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

Highlights:
1. This study was the first study identifying the characteristics of aphasia among ischemic stroke patients during COVID-19 pandemic at a national brain center in Indonesia.
2. This study provides additional data for future studies to conduct a comparison between the pre- and post-pandemic incidence of aphasia.

INTRODUCTION

Stroke is an acute neurological condition that became the third-most common cause of disability worldwide in 2013. In terms of causes of mortality, stroke ranked second globally in the same year. Stroke patients may experience focal and global neurological deficits due to vascular impairments (Feigin et al. 2017). The worsening of neurological symptoms may occur within the first 24 hours or later since the onset of the symptoms.

The prevalence of stroke has been known to increase with age. According to the 2018 Basic Health Research, 10.9% of Indonesians have experienced stroke. Stroke diagnosis rates were lowest in the 15–

24 age group (0.2%) and highest in those over 75 years old (43.1%). However, stroke incidence has been showing an increase among individuals under the age of 45 (Ministry of Health of the Republic of Indonesia 2014, 2019a).

There was an increase in the number of stroke cases during the COVID-19 pandemic. The mortality rate for patients with ischemic stroke increased due to the impact of COVID-19 infections, particularly in severe cases (Mubasher et al. 2021, Utomo 2022). The COVID-19 infection triggers ischemic strokes through several mechanisms. The three main mechanisms are the hypercoagulable state, vasculitis, and cardiomyopathy (Spence et al. 2020).

Following an incidence of stroke, the patient may experience aphasia. Approximately 12% of stroke survivors are affected by aphasia (Giulio 2020, Mitchell et al. 2021). Aphasia is the disruption of expressive or receptive language that arises due to damage to certain areas of the brain. This condition is classified according to the location of the causative lesion and the resulting clinical manifestations. The classification comprises global aphasia, motor aphasia, sensory aphasia, conduction aphasia, anomic aphasia, and transcortical aphasia (Hasanah 2017).

In East Jakarta, Indonesia, there is a national referral hospital known as the National Brain Center Prof. Dr. dr. Mahar Mardjono Hospital that specializes in treating patients with nervous disorders, specifically stroke. According to the 2018 data from the hospital's annual report, ischemic stroke was the most frequent disease treated in the emergency room. Out of 6,281 patients, 2,730 (43.4%) suffered from stroke, with 2,082 (76.3%) diagnosed with ischemic stroke. Secondary preventive measures play a crucial role in reducing the incidence of neurological deficits. Such measures can be implemented by comprehending the characteristics of aphasia in ischemic stroke patients (Kleindorfer et al. 2021). Therefore, the purpose of this study was to identify the characteristics of aphasia in ischemic stroke subjects at the National Brain Center Prof. Dr. dr. Mahar Mardjono Hospital, Jakarta, Indonesia, during the COVID-19 pandemic.

MATERIALS AND METHODS

This study was conducted at the National Brain Center Prof. Dr. dr. Mahar Mardjono Hospital, Jakarta, Indonesia, in October 2022. This descriptive study used a cross-sectional design with a total sampling technique for obtaining research samples (Setia 2016). Ischemic stroke patients with aphasia served as the research subjects for this study. All subjects gave consent to be involved in this research.

The inclusion criteria for the research subjects were ischemic stroke patients who experienced aphasia, were over the age of 18, and had a diagnosis through a computed tomography scan (CT scan) and/or magnetic resonance imaging (MRI) between January 1 and December 31, 2021. Patients who had a history of brain trauma and/or neurological conditions other than ischemic stroke were excluded from this study (Vilela & Rowley 2017).

A neurologist obtained the data through examinations during ward rounds and history-taking. A univariate analysis was performed using IBM SPSS Statistics for Mac, Version 25.0 (IBM Corp., Armonk, N.Y., USA) to calculate the frequency, proportion, and mean of the data. The types of aphasia were identified according to the Boston classification of aphasia (Fong et al. 2019).

RESULTS

Out of the 240 patients, 162 subjects met the inclusion criteria for data analysis after 42 patients were excluded. Table 1 shows the general characteristics of the research subjects. The subjects' ages ranged from 34 to 87 years, with a median age of 59.82 years. As many as 60 subjects (37%) were in the 55–65 age group and made up the majority of the samples. We found that male patients (97 subjects; 59.9%) experienced aphasia in ischemic stroke at a higher prevalence rate than female patients (65 subjects; 40.1%). The most common risk factors identified in this study were diabetes mellitus (50.0%), hypertension (90.1%), and dyslipidemia (759.9%). A history of stroke (33.3%), coronary heart disease (22.2%), atrial fibrillation (5.6%), and increased blood viscosity (17.3%) were other risk factors identified in this study. However, data on the patients' smoking status were not available for 80.9% of the subjects.

This study documented several aphasia therapeutic modalities, including rehabilitation, brain stimulation, and pharmacological treatment. A total of 138 subjects (85.2%) received rehabilitation, while the other 24 subjects (14.8%) did not. This might be due to the subjects' poor condition, which prevented them from performing the required exercises. No subject received transcranial magnetic stimulation (TMS). Nearly all of the subjects, with as many as 159 individuals (98.1%), received antiplatelets. Only 31 subjects (19.1%) received anticoagulants. Neuroprotectors (e.g., citicoline and piracetam) were only administered to five subjects (3.0%).

Table 1. General characteristics of the ischemic stroke patients with aphasia.

Categories	n (162)	%
Age (years)		
<55	51	31.5
55–65	60	37.0
66–75	36	22.2
>75	15	9.3
Sex		
Male	97	59.9
Female	65	40.1
BMI		
Underweight	2	1.2
Normal	60	37.0
Overweight	27	16.7
Class I obese	42	25.9
Class II obese	20	12.3
Not available	11	6.8
Hypertension		
Yes	146	90.1
No	16	9.9
Diabetes mellitus		
Yes	81	50.0
No	81	50.0
Dyslipidemia		
Yes	123	75.9
No	39	24.1
Stroke history		
Yes	54	33.3
No	108	66.7
Ischemic heart disease		
Yes	36	22.2
No	126	77.8
Atrial fibrillation		
Yes	9	5.6
No	153	94.4
Hypercoagulable state		
Yes	28	17.3
No	134	82.7
Smoking status		
Active smoker	25	15.4
Non-smoker	4	2.5
Ex-smoker	2	1.2
Not available	131	80.9
Rehabilitation		
Yes	138	85.2
No	24	14.8
Brain stimulation		
Yes	0	0.0
No	162	100.0
Pharmacological treatment		
Anticoagulant	31	19.1
Antiplatelet	159	98.1
rTPA	2	1.2
Neuroprotector	5	3.0

Notes: BMI=body mass index; rTPA=recombinant tissue plasminogen activator.

Table 2 displays the distribution of aphasia types among the research subjects. Global aphasia was the predominant type of aphasia among the subjects, with a prevalence of 71 cases (43.8%). The second and third most common types were motoric aphasia (54 subjects; 33.3%) and sensory aphasia (20 subjects; 12.3%), respectively. Aphasia types with a rare distribution in this study were transcortical motor aphasia (8 subjects; 4.9%), sensory transcortical aphasia (7 subjects; 4.3%), and anomic aphasia (2 subjects; 1.2%).

Table 2. Distribution of aphasia types in ischemic stroke subjects.

Aphasia types	Total	
	n	%
Sensory	20	12.3
Motor	54	33.3
Transcortical sensory	7	4.3
Transcortical motor	8	4.9
Global	71	43.8
Anomic	2	1.2
Total	162	100.0

Table 3 presents the distribution of lesion locations among the aphasic subjects. The lesion locations were organized and classified according to the three main parts of the brain and its lobes, as shown in Table 4. It was found that the temporal lobe was the most common location of the causative lesion in sensory aphasia cases. In the meantime, the frontal lobe, corona radiata, and frontal lobe were the primary locations of causative lesions in motor aphasia, transcortical motor aphasia, and global aphasia cases, respectively. The transcortical sensory aphasia cases revealed that the frontal, parietal, and temporal lobes were the three areas most frequently affected. In the anomic aphasia cases, the frontal and parieto-temporal lobes were the two most often damaged areas.

In subjects with sensory aphasia and an ischemic stroke history, the lesions were identified in the temporal lobe (15 subjects), parietal lobe (15 subjects), frontal lobe (8 subjects), and occipital lobe (2 subjects). In addition, one subject with a cerebellar lesion and one subject with a brain stem lesion both had sensory aphasia. Subjects with motor aphasia exhibited lesions in the parietal lobe (47 subjects), frontal lobe (36 subjects), temporal lobe (25 subjects), occipital lobe (15 subjects), cerebellum (6 subjects), and brain stem (5 subjects). Subjects with transcortical sensory aphasia had lesions in the parietal lobe (6 subjects), temporal lobe (5 subjects), frontal lobe (3 subjects), occipital lobe (2 subjects), cerebellum (1 subject), and brain stem (1 subject). Subjects with transcortical motor aphasia showed lesions in the parietal lobe (7 subjects), frontal lobe (3 subjects), temporal lobe (3 subjects), occipital lobe (3 subjects), and cerebellum (1 subject). Furthermore, subjects with global aphasia demonstrated lesions in the parietal lobe (66 subjects), frontal lobe (58 subjects), temporal lobe (47 subjects), occipital lobe (27 subjects), brain stem (7 subjects), and cerebellum (4 subjects).

Table 3. Distribution of the lesion locations among the subjects.

Lesion locations	Aphasia types (n)					
	Sensory	Motor	Transcortical sensory	Transcortical motor	Global	Anomic
Corona radiata	5	24	1	4	33	0
Frontal lobe	3	26	2	2	38	1
External capsule	3	9	0	2	7	0
Basal ganglia	1	21	1	3	29	0
Temporo-parieto-occipital lobe	0	1	1	0	0	0
Posterior insula	3	1	0	0	3	0
Fronto-parieto-temporal lobe	0	2	0	0	10	0
Cerebral peduncle	0	0	0	0	1	0
Parieto-temporal lobe	4	1	1	1	3	1
Parietal lobe	3	7	2	1	11	0
Temporal lobe	7	4	2	1	10	0
Fronto-parietal lobe	0	3	0	0	4	0
Parieto-occipital lobe	0	2	0	1	3	0
Pons	0	5	1	0	6	0
Insula	3	10	1	0	17	0
Occipital lobe	0	2	0	2	4	0
Cerebellum	1	6	0	1	4	0
Corpus callosum	0	2	0	0	1	0
Internal capsule	1	7	1	0	9	0
Anterior insula	0	1	0	0	0	0
Fronto-temporal lobe	1	1	0	1	5	0
Lateral periventricular	2	7	1	0	16	0
Centrum semiovale	0	3	1	1	4	0
Thalamus	1	11	0	0	8	0
Temporo-occipital lobe	0	3	0	0	2	0
Caudate nucleus	1	3	1	0	2	0
Lenticular nucleus	1	0	0	0	3	0
Fronto-temporo-parieto-occipital lobe	0	0	0	0	1	0
Watershed area	0	0	0	0	1	0

As shown in Table 4, we found lesions in the frontal lobe (1 subject), temporal lobe (1 subject), and parietal lobe (1 subject) of the research subjects who experienced anomic aphasia. These results demonstrated the categorical data synthesized from the more detailed lesion location distribution. Among the subjects with anomic aphasia, one individual had a temporoparietal lobe lesion, while the other individual had a single frontal lobe lesion.

Table 4. Distribution of lesion locations according to the main parts of the brain.

Lesion locations	Aphasia types (n)					
	Sensory	Motor	Transcortical sensory	Transcortical motor	Global	Anomic
Cerebrum						
Frontal lobe	8	36	3	3	58	1
Temporal lobe	15	25	5	3	47	1
Parietal lobe	15	47	6	7	66	1
Occipital lobe	2	15	2	3	27	0
Cerebellum	1	6	1	1	4	0
Brain stem	1	5	1	0	7	0

DISCUSSION

General characteristics of ischemic stroke patients with aphasia

The findings from our study were consistent with those of earlier studies. It was previously found that the average age of aphasic patients was 55.8 years old. The patients were between the ages of 50 and 60 (Fitri & Lastri 2019, Khedr et al. 2021). The risk of stroke has been shown to increase as people age. This is because of the thickening and loss of

elasticity of the tunica intima in blood vessels, which causes a decrease in the blood flow of the brain (Sofyan et al. 2013).

A previous study conducted by Hasanah (2017) showed that aphasia with an acute stroke history was more common in men (10 subjects; 58.8%) than in women (7 subjects; 41.2%). The incidence of stroke was also higher in men than women, even at younger ages. Nonetheless, the incidence of stroke in women increases with age due to the neuroprotective effect of estrogen on the brain (Bushnell et al. 2018).

The results of our study were in line with previous investigations regarding the risk factors for aphasia. Hypertension was present in 72.0% of aphasic patients (Couto et al. 2020). A substantial number of aphasic patients also had diabetes mellitus (91.25%), while a smaller number of the patients experienced dyslipidemia (34.9%), atrial fibrillation (37%), and coronary heart disease (26%) (Grönberg et al. 2022). Meanwhile, a study conducted by Rasyid et al. (2019) examined the risk factors for ischemic stroke. They found that blood viscosity was prevalent in 88.6% of ischemic stroke patients.

Smoking has a major impact on stroke incidence. It was found that the risk of stroke increases by 12% for every five cigarette butts consumed per day. Smoking has been associated with atherosclerosis, changes in homocysteine, fibrinogen, and oxidized low-density lipoprotein cholesterol levels. This activity has also demonstrated an association with atrial fibrillation, diabetes mellitus, and hypertension, which are risk factors for stroke (Pan et al. 2019).

In this study, aphasia following an ischemic stroke was more common in overweight individuals. This finding agreed with a previous study conducted by Nabila et al. (2020), who found that ischemic stroke was most prevalent in individuals within the body mass index (BMI) range of 25.0-29.9. Blood lipid imbalance is often associated with excess body weight. It has been demonstrated that obesity can lead to atherosclerosis and increased stroke incidence (Mitchell et al. 2015).

Previous studies indicate that the best time to initiate anticoagulant therapy in ischemic stroke patients has not yet been established, and it requires further research. It is also not recommended to use antiplatelets in combination with anticoagulants as a preventive treatment for secondary stroke (Froio et al. 2017, Kleindorfer et al. 2021). According to the guidelines issued by the Ministry of Health of the Republic of Indonesia (2019b), recombinant tissue plasminogen activator (rTPA) therapy can be administered to ischemic stroke patients with ≤6 hours of symptom onset. Out of 162 subjects in this study, only two patients (1.2%) received rTPA therapy. One research subject was admitted to the hospital with 3 hours of symptom onset, while the other subject came with 30 minutes of symptom onset. The national guidelines recommend the administration of neuroprotectors for stroke patients who have not received reperfusion therapy. However, citicoline and piracetam were not included in the national formulary. This probably led to a decrease in the number of ischemic stroke patients receiving these medications.

Distribution of aphasia types in ischemic stroke patients

We found that the distribution of aphasia types in patients with ischemic stroke was consistent with other studies. Global aphasia was found to be the most common aphasia in the studies conducted by Lee et al. (2018) and Grönberg et al. (2022). In a separate study conducted by Fitri & Lastri (2019), motor aphasia was the most prevalent type of aphasia, with as many as four patients (28.6%). Three patients (21.4%) had global aphasia, making it the second most prevalent type of aphasia in the study.

Lesion locations in patients with sensory aphasia

The parietal lobe is composed of several structures, including the corona radiata, basal ganglia, thalamus, external and internal capsules, and caudate and lentiform nuclei. The corpus callosum and insula lie in three lobes (i.e., frontal, parietal, and temporal lobes), while the lateral periventricle and centrum semiovale are located in every lobe of the brain (i.e., frontal, parietal, temporal, and occipital lobes). In this study, corpus callosum lesions were categorized according to the parts of the corpus callosum affected (from the genus of the corpus callosum to the frontal lobe). A previous study showed that the causative lesions of sensory aphasia were located in the parietotemporal lobe, temporal lobe, basal ganglia, and lentiform nucleus, as well as the external and internal capsules (Khedr et al. 2021, Duron et al. 2022).

Another name for sensory aphasia is Wernicke's aphasia, which refers to a lesion location known as the Wernicke's area. This area covers the superior temporal gyrus, at the junction of the parietal lobe and the temporal lobe (Døli et al. 2021). While lesions of sensory aphasia are typically found in the Wernicke's area, three aphasic subjects in our study showed evidence of lesions in the frontal lobe as well. In addition, lesions in the basal ganglia, temporal lobe, parietal lobe, and corona radiata of the subjects corroborated the clinical manifestations of sensory aphasia.

In this study, subjects who had lesions in their thalamus also exhibited lesions in their left temporal lobe. As a result, it was possible that there would be overlap between the symptoms of thalamic and sensory aphasia. Thalamic aphasia is characterized by impairments in verbal fluency and comprehension. However, this type of aphasia may also not affect comprehension and repetition abilities in certain cases (Rangus et al. 2022).

The superior cerebellum is involved in the activation of articulations and verbal memory. Nonetheless, lesions in the cerebellum have been associated with motor aphasia Jianu et al. (2022). One subject in our study who experienced sensory aphasia, however, had both cerebellum and temporo-parietal lobe lesions. This might be because temporo-parietal lobe lesions are more closely associated with sensory aphasia. The cerebellum, basal ganglia, and thalamus comprise the cortico-subcortical neural network, which is involved in planning, coordinating, timing, sequencing, and selecting the language production process. The interaction of the fronto-striatal and fronto-temporal pathways involves the basal ganglia in language production. Dysfunction of the circuit can reduce the efficiency of word selection. The integration of the cerebellum, basal ganglia, and thalamus also regulates repetition and speech block. Therefore, disturbances in these areas can lead to stuttering (Silveri 2021).

The insula plays an important role in language processing. Damage to the insula is often associated with motor aphasia, word-deafness in sensory aphasia, and repetition difficulty in conduction aphasia (Ardila 2018). Our study found similar lesions in the insula of subjects who experienced various types of aphasia, including sensory aphasia.

Lateral periventricular area is related to automatic speech production. However, Jianu et al. (2022) revealed that lateral periventricular lesions were frequently associated with motor aphasia. In this study, lesions were found in the lateral periventricular area of two subjects. These subjects also exhibited lesions in the temporal lobe, which corresponded to the clinical manifestation of sensory aphasia.

In this study, one subject each had a lesion in the lenticular nucleus and caudate nucleus. A previous study found that lesions in the caudate and lenticular nuclei were prevalent in patients with sensory aphasia (Bohra et al. 2015). The expansion of the caudate nucleus enhances language switching. The nucleus also works with the thalamus in the process of language selection. In addition, the basal ganglia, which has a role in language processing, is composed of the caudate and lenticularis nuclei (Booth et al. 2007).

According to a prior study conducted by Jianu et al. (2022), posterior insula lesions are more likely to be associated with motor aphasia. However, three subjects who had sensoric aphasia in this study exhibited lesions in the posterior insula. Two of the three subjects also had lesions in the parietotemporal lobe, while the third subject had a lesion in the temporal lobe, which was associated with sensory aphasia. There has been no established association yet between sensory aphasia and lesions in the corona radiata. Therefore, lesions found in the corona radiata of five subjects in this study might have a stronger association with the lesions found in the caudate nucleus, temporal lobe, parietal lobe, and temporo-parietal lobe.

Lesion locations in patients with motor aphasia

Broca's complex and its borders (i.e., dorsolateral prefrontal cortex, supplementary motor area, basal ganglia, and insula) have a role in language processing. Lesions in the anterior insula cause difficulty in verbal fluency (Ardila 2018). Damage to the medial temporal lobe is associated with the auditory comprehension process. Meanwhile, lesions in the frontal lobe and insula may affect repetition ability (Døli et al. 2021). Eleven subjects who had motor aphasia in this study exhibited thalamic lesions accompanied by lesions in the frontal lobe, basal ganglia, and corona radiata in other parts of the lobe, which corresponded to the clinical manifestations of motor aphasia. Lesions in both Broca's area and cerebellum have been associated with severe motor aphasia (Lorca-Puls et al. 2021). In our study, there were six subjects with cerebellum lesions.

According to a study conducted by Flowers et al. (2013), pontine lesions generally do not cause aphasia. Two of the five subjects in our study who had pontine lesions additionally exhibited frontal lobe lesions. The remaining three subjects also showed the presence of lesions in the basal ganglia (2 subjects) as well as the corona radiata and thalamus (1 subject).

Lesions in the corpus callosum have been reported to cause aphasia symptoms. Symptoms of expressive aphasia may arise from infarctions of the corpus callosum and corona radiata (Lan et al. 2020). Corpus callosum activity can suppress cortical activity in the opposite hemisphere. The absence of callosal fiber can lead to interference in the left hemisphere, which in turn suppresses activity in the language areas of the opposite hemisphere (Hinkley et al. 2016)

We identified several non-specific lesions in the parieto-temporal, parietal, temporal, and parieto-occipital lobes of subjects with motor aphasia.

However, it has been discovered that these areas are more specific for sensory aphasia (Khedr et al. 2021). The different results of this study were probably caused by overlapping lesion locations or subjectivity in expert clinical assessment. Fluctuations in cerebral blood flow could also possibly contribute to these results (Walenski et al. 2022).

Lesion locations in patients with transcortical sensory aphasia

Transcortical sensory aphasia has been associated with lesions on the insula, parietal lobe, temporal lobe, frontal lobe, internal and external capsule, caudate nucleus, and basal ganglia. The core location of lesions in transcortical sensory aphasia is the watershed area. This site is located in the dominant hemisphere cortex between the parietal and temporal cortices, near Wernicke's area (Kasselimis et al. 2011, Wang et al. 2021).

In this study, subjects with transcortical sensory aphasia exhibited pontine lesions, accompanied by lesions in other locations, including the temporo-parietal lobe, internal capsule, caudate nucleus, lateral periventricular area, and centrum semiovale. In addition, subjects who had corona radiata lesions also exhibited lesions in the parietal lobe. This indicated the presence of clinical manifestations of transcortical sensory aphasia (Kasselimis et al. 2011).

Lesion locations in patients with transcortical motor aphasia

Transcortical motor aphasia has been identified as being caused by lesions in the frontal lobe. The location of the causative lesion includes the watershed area in the dominant hemisphere between the frontal lobe and the parietal lobe, as well as the anterior and superior regions of Broca's area. In addition, transcortical motor aphasia has also been associated with lesions in the centrum semiovale (Wang et al. 2021, Jianu et al. 2022).

To the best of the authors' knowledge, there has been no established association yet between transcortical motor aphasia and several lesion locations, including the parieto-temporal, parietal, temporal, parieto-occipital, and occipital lobes. Two of the research subjects with transcortical motor aphasia had lesions in the corona radiata, basal ganglia, and occipital lobe. Lesions were also found in the parieto-occipital and frontal lobes of one subject, in the temporal and frontal lobes of one additional subject, and in the cerebellum and frontal lobes of another subject. Furthermore, two subjects exhibited lesions in the external capsule, corona radiata, and basal ganglia. Subjects with lesions in the centrum semiovale had other lesions in the corona radiata, external capsule, basal ganglia, and temporal lobe. This confirmed the clinical manifestations of transcortical motor aphasia (Li et al. 2021). However, subjects with lesions in the temporo-parietal lobe and the parietal lobe only had a single lesion each. This was probably due to the subjectivity of the attending doctor.

Lesion locations in patients with global aphasia

Global aphasia is caused by large lesions that can be found in numerous locations. The lesion locations include the frontal lobe, parietal lobe, occipital lobe, insula, internal capsule, basal ganglia, thalamus, caudate and lentiform nuclei, corpus callosum, centrum semiovale, and corona radiata (Bohra et al. 2015, Kang et al. 2017, Krishna Karthik et al. 2017). As previously discussed, pontine lesions generally do not cause aphasia. However, six subjects in our study exhibited pontine lesions, accompanied by other lesions that are associated with global aphasia, i.e., the basal ganglia, frontal lobe, parietal lobe, insula, thalamus, corona radiata, external capsule, and fronto-parieto-temporal lobe.

In this study, four subjects had lesions in the cerebellum. These subjects also exhibited lesions in other locations associated with global aphasia, including the basal ganglia, corona radiata, and fronto-parieto-temporo-occipital lobe, frontal lobe, parieto-occipital lobe, and insula. In the meantime, lesions in the watershed area are more often associated with transcortical aphasia (Khedr et al. 2021). One of our research subjects, however, had a watershed area lesion along with lesions in other locations associated with global aphasia. The occipital lobe, parietal lobe, and corona radiata were among the lesion locations.

The periventricular area has been associated with automatic speech production, as was previously discussed. In contrast, it was discovered that motoric aphasia was more frequently linked to lesions in the lateral periventricular area (Jianu et al. 2022). Subjects in our research who had lesions in the lateral paraventricular area also had lesions in additional locations related to global aphasia. We have not found any association between global aphasia and lesions in the cerebral peduncle and occipital lobe from prior studies. However, our research subjects who exhibited cerebral peduncle lesions also had lesions in the fronto-parieto-temporal lobe, which is associated with global aphasia. The subjects who had occipital lobe lesions also had other lesions in other locations associated with global aphasia. The parietal, frontal lobe, basal ganglia, posterior insula, corona radiata, temporal lobe, external capsule, and lateral periventricular were among the locations of the lesions.

Lesion locations in patients with anomic aphasia

The result of our study confirmed a study conducted by Silva et al. (2020) that lesions in the temporal and parietal lobes are the underlying cause of anomic aphasia. Our research subjects who had anomic aphasia exhibited lesions in the temporal and parietal lobes. One of the subjects who had anomic aphasia also exhibited a lesion in the frontal lobe. However, there has been no established association between anomic aphasia and frontal lobe lesions. The subjectivity of the attending doctor might result in contrast findings compared to those of earlier research.

Strength and limitations

This study can provide additional data on the various types of aphasia during the COVID-19 pandemic. The findings of this study may be useful for further studies to examine the prevalence of aphasia pre- and post-pandemic. However, this study encountered limitations due to the patients' incomplete data, affecting the sample size and the different clinical interpretations of the aphasia types between the emergency room physician and the attending neurologist at the ward. The diagnosis from the attending neurologist served as the basis for the data used in this study.

CONCLUSION

Global aphasia was the most prevalent type of aphasia in this study. The causative lesions were most frequently found in the parietal lobe. The majority of ischemic stroke patients with aphasia were middle-aged men. The primary risk factors for the disorder were hypertension, diabetes, and dyslipidemia. Additionally, aphasia was often observed in ischemic stroke patients who were overweight.

Acknowledgment

We would like to thank all the staff at the Department of Neurology of the National Brain Center Prof. Dr. dr. Mahar Mardjono Hospital, Jakarta, Indonesia, for their contribution to this study.

Conflict of interest

None.

Ethical consideration

This study was approved by the Research Ethics Committee of Universitas Pembangunan Nasional "Veteran" Jakarta, Jakarta, Indonesia, with reference No. 381/IX/2022/KEPK on 1/9/2022.

Funding disclosure

None.

Author contribution

RH contributed to the conception and design, analysis and interpretation of the data, drafting of the article, provision of study materials or patients, statistical expertise, acquisition of funding, provision of administrative, technical, or logistic support, and the collection and assembly of data. AY, RM, and RV were involved in the conception and design, critical revision of the article for important intellectual content, and final approval of the article.

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Original Research Report

AN IN VITRO ASSAY REVEALS THE ANTI-AGING PROPERTIES OF TEMULAWAK EXTRACT (*Curcuma xanthorrhiza* L.)

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ABSTRACT

Aging is the gradual loss of a tissue's capacity to heal and maintain normal or physiological form and function. Elastase, hyaluronidase, and tyrosinase are the enzymes that contribute to the process of skin aging. The anti-aging effect is connected to the inhibition of these enzymes' activities. Numerous medicinal plants with active metabolites have been extensively utilized to treat aging. The active compounds of temulawak (*Curcuma xanthorrhiza* L.), such as phenolics, curcuminoids, and xanthorrhizol, have promising properties that may be used as anti-aging agents. This study aimed to determine whether temulawak extract, a potential option for cosmeceuticals, has anti-aging properties that can inhibit the enzymes elastase, hyaluronidase, and tyrosinase. A stock solution was made by dissolving 20 mg of temulawak extract in 1 mL of 100% dimethyl sulfoxide (DMSO). The stock solution was then diluted to produce working solutions with concentrations ranging from 31.25 to 1000 µg/mL. An in vitro assay was carried out in three replications to examine the anti-aging activity of the temulawak extract. The in vitro assay investigated the inhibition of the enzyme elastase, hyaluronidase, and tyrosinase at seven different concentrations, with the following ranges: 2.08–66.67 µg/mL for the anti-elastase, 5.21–166.67 µg/mL for the anti-hyaluronidase, and 3.125–100 µg/mL for the anti-tyrosinase. IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, N.Y., USA) was used to perform the statistical analysis, with a significance level of $p<0.05$. Temulawak extract exhibited the highest inhibition rates, reaching 82.72%, 89.41%, and 94.17% for the anti-tyrosinase, anti-elastase, and anti-hyaluronidase activities, respectively. The median inhibitory concentrations (IC_{50}) were 10.66, 70.39, and 55.87 µg/mL for the elastase, hyaluronidase, and tyrosinase activities, respectively. This study revealed that temulawak extract has strong anti-aging properties as it effectively inhibits the activities of elastase, tyrosinase, and hyaluronidase. In conclusion, temulawak extract can be considered a promising candidate for cosmeceutical applications.

Keywords: Anti-aging cosmeceutical; anti-elastase; anti-hyaluronidase; *Curcuma xanthorrhiza*; healthy lifestyle

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Article history
●Submitted 20/8/2023 ● Revised 4/10/2023 ● Accepted 13/11/2023 ● Published 10/12/2023

How to cite: Wargasetia TL, Widowati W, Muthmainnah AS, et al (2023). An in Vitro Assay Reveals the Anti-Aging Properties of Temulawak Extract (*Curcuma xanthorrhiza* L.). Folia Medica Indonesiana 59 (4), 352-358, https://doi.org/10.20473/fmi.v59i4.44944

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

Highlights:
1. This study investigated the potential of temulawak extract as a cost-effective option for cosmeceutical applications in the treatment of aging.
2. Temulawak extract was found to have the ability to inhibit elastase, hyaluronidase, and tyrosinase activities, thus making it a suitable option for cosmeceutical applications.

INTRODUCTION

Age-related illnesses and geriatric syndromes, such as obstructive lung conditions, musculoskeletal issues, different types of cancer, neurological conditions, and skin conditions, are brought on by the gradual decline in physiological function that accompanies the aging process. The continuous reduction of the structural integrity and physiological function of the skin is caused by a variety of internal and external factors that affect skin aging (Popoola et al. 2015, Costa et al. 2022). All people experience natural processes that lead to aging, which are caused by intrinsic factors. Clinical alterations associated with intrinsic aging include decreased skin barrier function, increased vascularization of the skin layer, and reduced epidermal cell turnover, which result in skin

atrophy. Skin protection, absorption, excretion, secretion, thermoregulation, and sensory perception are consequently compromised. The ultraviolet (UV) spectrum has an impact on the occurrence of extrinsic skin aging. Wrinkles, hypopigmentation, hyperpigmentation, rough skin, loss of skin color, dryness, and melanoma are clinical symptoms resulting from external factors (Rihhadatulaisy & Putriana 2020).

Following exposure to photoaging stimuli, the skin accumulates reactive oxygen species (ROS), which can indirectly activate dermal enzymes such as elastase. The dermal enzyme activation effectively breaks down and degrades elastin (Popoola et al. 2015, Chatatikun & Chiabchalard 2017). Therefore, elastase synthesis promotes premature aging of the skin. Such aging is characterized by the presence of distinct symptoms such as deep furrows, freckles, sallowness, laxity, wrinkles or severe atrophy, and leathery texture (Ding et al. 2018). Hyaluronic acid, commonly known as hyaluronan (HA), is a glucose-based polymer found in the body's tissues and fluids. However, it is predominantly concentrated in the epidermal layer and dermal compartment of the skin. HA primarily aids in skin renewal, provides moisture, thickens fluids, and lessens extracellular fluid permeability (Jiratchayamaethasakul et al. 2020).

Because of the superior water-holding capacity of HA, areas rich in this substance have enhanced emollience, smoothness, and youthfulness, as well as reduced wrinkles. In contrast to hyaluronidase, which is normally produced as we age, HA regrettably and naturally diminishes. The skin ages due to the loss of strength, flexibility, and moisture caused by the HA-destructing enzyme hyaluronidase (Ndlovu et al. 2013, Jegasothy et al. 2014). One of the methods to combat wrinkles is by preserving the HA contents under the skin to prolong skin moisture. Tyrosinase, a melanogenic enzyme, plays a crucial role because it can limit the rapid rate of melanin-based coloration. Hence, a typical strategy for pigmentation disorder treatment and whitening for aesthetic purposes is the suppression or inhibition of tyrosinase activity (Chatatikun & Chiabchalard 2017, Kang et al. 2018).

The application of cosmetic products helps to enhance the natural beauty and appeal of the skin while protecting it against harmful effects from both external and internal factors. By minimizing skin disorders, cosmetic products not only enhance the surface appearance of the skin but also extend the longevity of healthy skin. Skin care products maintain the suppleness of the skin by reducing type I collagen and providing photoprotection, among other benefits, while simultaneously nourishing its health, texture, and integrity. The use of synthetic or natural substances in skin care formulations contributes to the traits of the resulting cosmetic products, including their abilities to reduce the visibility of free radicals on the skin and manage their properties over an extended period of time. Cosmetics are one of the best alternatives for treating various skin conditions, such as hyperpigmentation, wrinkles, rough skin texture, and skin aging. The evaluation of the efficacy of chemicals present in skin care products encounters limitations that restrict the development of novel skin care formulations. A range of in vivo models are employed to assess the safety and effectiveness of cosmetic formulations, often involving the participation of human volunteers. The in vivo evaluation of cosmetic products on human volunteers presents a number of drawbacks, including high costs, lengthy duration, and potential risks to the human clinical subjects. Additionally, it can be exceedingly challenging to obtain human ethical clearance. As a result, there is growing interest in conducting in vitro procedures due to their reduced reliance on human volunteers, improved budgetary efficiency, and time-saving benefits. Using an in vitro model to assess the efficacy of skin care formulations can also potentially reduce the price of the final products (Sahu et al. 2013).

Dermatologists currently have a variety of options for anti-aging treatments, each with its own advantages and disadvantages. Customers commonly seek non-invasive cosmetics and skincare products that are both safe and effective due to concerns over their health and well-being (Ahmed et al. 2020). Plant species offer an abundant supply of raw materials, which enables the production of standardized herbal products. This production process requires scientific assessments to determine the efficacy, safety, and quality control of the materials. These materials may be beneficial in the management and prevention of various disorders, particularly those associated with skin aging. Temulawak, scientifically known as *Curcuma xanthorrhiza* L., is one of the plants that possesses anti-aging properties. Temulawak is composed of the active ingredients curcuminoid and xanthorrhizol. Curcumin, a natural dietary polyphenol, has exhibited diverse biological and pharmacological effects, including anti-aging benefits. Meanwhile, a potential anti-aging compound known as xanthorrhizol has shown its ability to dramatically lower metalloproteinase-1 (MMP-1) expression and boost type 1 procollagen production (Vaiserman et al. 2020, Irfan et al. 2021). Therefore, this study aimed to ascertain whether temulawak extract, a potential candidate for a cosmeceutical ingredient, has anti-aging properties that can inhibit the enzymes elastase, hyaluronidase, and tyrosinase.

MATERIALS AND METHODS

The reagents and chemicals used for the elastase inhibitory assay were n-sucanyl-ala-ala-ala-p nitroanilide (Sigma-Aldrich S4760-25MG, Burlington, USA), elastase from porcine cell culture (HiMedia TC311-10MG, Maharashtra, India), tris (BioRad Cat. #1610716, Hercules, USA), sodium chloride (Merck 1.06404.1000, Darmstadt, Germany), distilled water, dimethyl sulfoxide (Merck 1.02952.1000, Darmstadt, Germany), and hydrochloric acid solution (Merck 1.00317.1000, Darmstadt, Germany).

In the hyaluronidase inhibitory assay, the reagents and chemicals used were sodium phosphate monobasic (1.06346.1000, Merck, Darmstadt, Germany), hyaluronic acid sodium salt (H5542-50MG, Sigma-Aldrich, Burlington, USA), cell-culture-tested grade hyaluronidase (TC331-25MG, HiMedia , Maharashtra, India), sodium chloride (1.06404.1000, Merck, Darmstadt, Germany), aquades, bovine serum albumin (A2153-100G, Sigma-Aldrich, Burlington, USA), sodium acetate (1.06268.1000, Merck, Darmstadt, Germany), acetic acid made in Indonesia by CV. Agung Menara Abadi (6°55'28.5"S 107°42'05.2"E), hydrochloric acid solution (1.00317.1000, Merck, Darmstadt, Germany), and sodium hydroxide (1.055.872.500, Merck, Darmstadt, Germany).

The reagents and chemicals utilized in the tyrosinase inhibitory assay were potassium dihydrogen phosphate (1.048.730.250, Merck, Darmstadt, Germany), dipotassium hydrogen phosphate (1.051.041.000, Merck, Darmstadt, Germany), mushroom tyrosinase (T3824-50KU, Sigma-Aldrich, Burlington, USA), l-3,4-dihydroxy-phenylalanine (L-DOPA) (D9628-25G, Sigma-Aldrich, Burlington, USA), potassium hydroxide (P5958-500G, Sigma-Aldrich, Burlington, USA), and distilled water.

Temulawak extract used in this study was produced by PT Fast (Depok, Indonesia) in compliance with Good Manufacturing Practices (GMP) with batch number 00110201069. The sample was initially prepared by adding 70% ethanol to dried temulawak, followed by the addition of lactose (Widowati et al. 2023). The stock solution was made by dissolving 20 mg of the extract in 1 mL of 100% dimethyl sulfoxide (DMSO) solvent. The stock solution was then diluted to achieve various concentrations of working solution, i.e., 31.25, 62.5, 125, 250, 500, and 1000 µg/mL.

By referring to the previous study conducted by Widowati et al. (2018), the evaluation of the inhibitory activity of elastase was conducted using a modified method. Elastase derived from porcine pancreas, with a concentration of 0.5 mU/mL in cold distilled water, was mixed with 135 µL of Tris buffer (100 mM, pH 8). The mixture was then combined with 10 µL of the samples, ranging in concentration from 2.08 to 66.67 µg/mL. The resulting mixture was pre-incubated for 15 minutes at 25 °C. After pre-incubating the mixture, 10 µL of the n-sucanyl-ala-ala-ala-p-nitroanilide substrate (2 mg/mL in Tris buffer) was added. Afterwards, the mixture was incubated at 25 °C for 15 minutes before measuring the absorbance at a wavelength of 410 nm using a microplate spectrophotometer (Multiskan™ GO microplate spectrophotometer, Thermo Fisher Scientific, USA). The proportion of elastase inhibitory activity was calculated using the following formula: inhibitory activity % = (A control - A sample)/ A control × 100. In this context, the A control represented the inhibitory activity observed in the group that did not receive temulawak extract, whereas the A sample referred to the inhibitory activity of temulawak extract.

According to the methods outlined in previous studies by Tu & Tawata (2015) and Widowati et al. (2018), the hyaluronidase inhibitory activity was assessed using an established technique from Sigma-Aldrich. Concisely, 25 µL of the samples (5.21–166.67 µg/mL) were pre-incubated at 37 °C for 10 minutes by mixing them with 3 µL of hyaluronidase (0.02 mg/mL in 20 mM phosphate buffer, pH 7, containing 77 mM sodium chloride and 0.01% bovine serum albumin) and 12 µL of phosphate buffer (300 mM, pH 5.35). After the pre-incubation, 10 µL of hyaluronic acid substrate (0.03% concentration in 300 mM phosphate buffer, pH 5.35) was then incubated at 37 °C for 45 minutes. The reaction was stopped by adding 100 µL of acidic albumin acid (24 mM sodium acetate, 79 mM acetate acid, and 0.1% bovine serum albumin). After 10 minutes at room temperature, the mixture was analyzed for absorbance at 600 nm. The following formula was used to determine the proportion of hyaluronidase inhibitory activity: inhibitory activity % = (A control - A sample)/ A control × 100. The A control signified the inhibitory activity in the group that was not given temulawak extract, while the A sample represented the inhibitory activity of temulawak extract.

Prior studies conducted by Tu & Tawata (2015) and Siregar et al. (2019) provided a reference for conducting a tyrosinase inhibitory assay. A modified procedure was employed to assess the inhibitory activity of tyrosinase. Briefly, 20 µL of the samples were mixed with 140 µL of potassium phosphate buffer (20 mM, pH 6.8) and 20 µL of mushroom tyrosinase (from a volume of 125 U/mL dissolved in potassium phosphate). Afterwards, the incubation was carried out for 15 minutes at room temperature. Following the incubation, 20 µL of L-DOPA (1.5 mM) was added. The resulting mixture was

incubated once more for 10 minutes at room temperature. The absorbance was measured using a spectrophotometer at 470 nm. The formula used for calculating the proportion of tyrosinase inhibitory activity was as follows: inhibitory activity % = (A control - A sample)/ A control × 100. The A control indicated the inhibitory activity of the group with no administration of temulawak extract. Conversely, the A sample represented the inhibitory activity of temulawak extract.

The statistical analysis was performed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, N.Y., USA). The results were presented as mean±standard deviation. The normality and homogeneity of the data were examined using the Shapiro-Wilk test and the Levene test, respectively. If the data did not exhibit a normal distribution, the Kruskal-Wallis test and Mann-Whitney test were employed to examine differences across the groups. If the data showed a normal distribution, a one-way analysis of variance (ANOVA) was conducted, followed by Tukey's honest significant difference (HSD) post-hoc test. Statistically significant differences were defined as those with $p \leq 0.05$ (Vetter 2017).

RESULTS

The temulawak extract was tested for its inhibitory effect against elastase, hyaluronidase, and tyrosinase. Figure 1 depicts the inhibitory effects of temulawak extract on elastase, hyaluronidase, and tyrosinase, presented as a percentage.

The temulawak extract was diluted in 10% DMSO to produce different ranges of concentrations to be used in three experiments. The first experiment used various concentrations of 2.08, 4.17, 8.33, 16.67, 33.33, and 66.67 µg/mL (Figure 1a). In the second experiment, the concentrations used were 5.21 µg/mL, 10.42, 20.83, 41.67, 83.33, and 166.67 µg/mL (Figure 1b). Lastly, the third experiment tested the following concentrations: 2.08, 4.17, 8.33, 16.67, 33.33, and 6667 µg/mL (Figure 1c). The results of these experiments indicated the differences in inhibitory activity of temulawak extract against elastase, hyaluronidase, and tyrosinase, respectively. The data collected from the experiments were presented as mean±standard deviation. The Mann-Whitney test was used in the first experiment, while Tukey's HSD post-hoc test was used in the second and third experiments to determine the statistical differences. Various letters in the diagrams (i.e., a, b, c, d, e, and f) signified the significant differences in inhibitory activity at different concentrations ($p<0.05$).

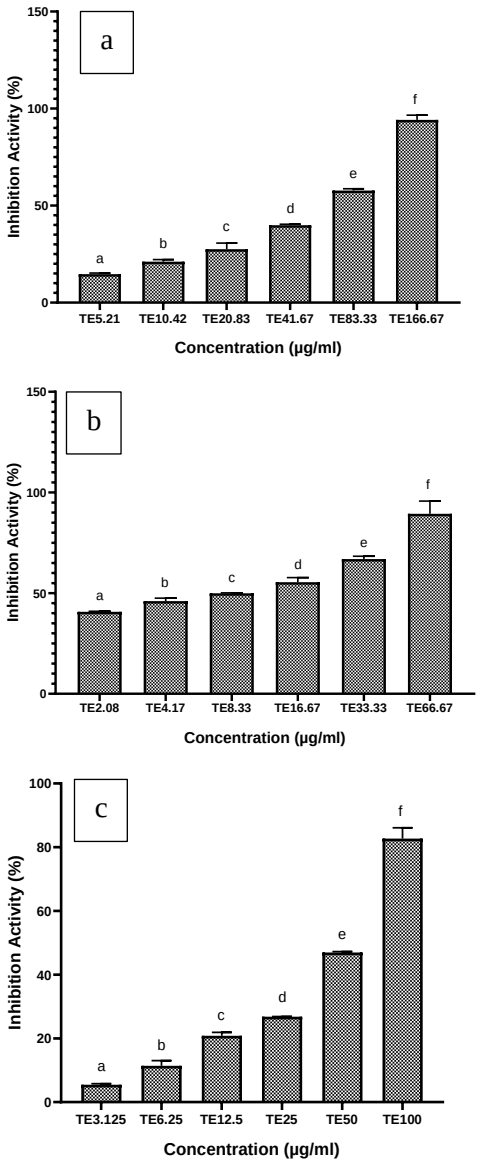

Figure 1. Different temulawak extract concentrations showed different levels of inhibitory effect on (a) elastase, (b) hyaluronidase, and (c) tyrosinase. Different superscripts (a, b, c, d, e, and f) show the significant difference among the treatments group ($p<0.05$).

The results demonstrated that there were inhibitions of elastase, hyaluronidase, and tyrosinase by the temulawak extract. The level of inhibition rose proportionally with the increasing concentration. Furthermore, the anti-aging inhibition was assessed by determining the median inhibitory concentrations (IC_{50}) of the samples, as exhibited in Table 1. The inhibition assays were carried out in triplicate. The coefficient of determination (R^2) indicated how well our data fit the regression model. The IC_{50} value of each sample was determined using linear regression. The temulawak extract showed the highest IC_{50} in inhibiting hyaluronidase and the lowest IC_{50} in

inhibiting elastase. Accordingly, the temulawak extract was the least potent hyaluronidase inhibitor and the most potent elastase inhibitor.

Table 1. The median inhibitory concentrations (IC_{50}) of temulawak extract.

Inhibition assays	R^2	Linear regression	IC_{50}
Elastase	0.99	y = 0.7163x + 42.394	10.62
Hyaluronidase	0.99	y = 0.4761x + 16.486	70.39
Tyrosinase	0.99	y = 0.7668x + 7.2013	55.81

Notes: R^2=coefficient of determination; IC_{50}=half-maximal inhibitory concentrations (µg/mL).

DISCUSSION

Chronic exposure to exogenous sources of ROS has been associated with melanogenesis, the formation of wrinkles, and the deterioration of the antioxidant system in the skin. Tyrosine, hyaluronic acid, and elastin are elements of the extracellular matrix (ECM) that are believed to be significantly affected by aging factors due to their susceptibility to oxidative damage caused by free radicals throughout the aging process. Excessive UV exposure leads to cellular damage, the production of ROS, inflammation, and angiogenesis, which in turn cause numerous skin alterations such as pigmentation, erythema, laxity, wrinkles, and skin cancer (Nurrochmad et al. 2018). Due to their medicinal capabilities, including the ability to slow down the aging of the skin, plants have been widely utilized to treat a variety of ailments. In order to identify the class of molecules that potentially have anti-aging properties, phytochemical examination of plants is necessary. A recent study showed that temulawak extract contains certain beneficial substances such as phenols, tannins, curcuminoids, and xanthorrhizol (Rahmat et al. 2021).

This study demonstrated that temulawak extract exhibited the strongest elastase inhibitory activity at a concentration of 66.67 µg/mL (89.41%), with an IC_{50} value of 10.66 µg/mL. Temulawak extract has been shown to possess very active anti-elastase properties, with an IC_{50} value below 50 µg/mL (Tu & Tawata 2015, Dewi et al. 2020). Elastase is a protease belonging to the chymotrypsin family. It is principally in charge of degrading elastin, which is essential for preserving skin suppleness. Elastin is a fibrose protein that accounts for 2–4% of the ECM and aids in maintaining skin hydration. Moreover, elastase accelerates the progression of inflammation, delays the healing of wounds, and serves as the primary enzyme that affects all of the main proteins in the connective tissue matrix (Azmi et al. 2014). The discovery of inhibitors for the elastase enzymes can help prevent the loss of skin elasticity and slow the aging process. The findings of this study suggest that the phenolic and curcumin components in temulawak extract may have anti-aging abilities through elastase inhibitory activity.

The proteolytic enzymes hyaluronidase and elastase, which are found in the dermis, are responsible for breaking down hyaluronan and elastin of the extracellular matrix, respectively. The visible indications of aging, such as wrinkles and sagging of the skin, are largely caused by the loss of elastin. This study demonstrated that temulawak extract exhibited remarkable anti-hyaluronidase activity at a concentration of 166.67 µg/mL (94.17%), with an IC_{50} value of 70.39 µg/mL. Previous studies have found that temulawak extract shows active anti-hyaluronidase properties with an IC_{50} value between 50 and 100 µg/mL (Tu & Tawata 2015, Dewi et al. 2020). The enzyme hyaluronidase aids in the breakdown of HA. As a glycosaminoglycan polymer that functions in the tissues, HA is a critical substance in the aging process. It acts as a molecule that enhances skin hydration by maintaining a consistent water balance. In addition, HA serves as a component of the ECM, which contributes to the preservation of the skin's suppleness. The breakdown of HA is catalyzed by the enzyme hyaluronidase, which breaks down the N-acetyl-D-glucosamine and D-glucuronic acid residues of HA through the hydrolysis of 1,4-hexosaminidic linkages (Azmi et al. 2014, Jusri et al. 2019). HA can be broken down enzymatically as well as non-enzymatically by free radicals in the presence of decreasing chemicals such as iron ions, ascorbic acid, copper ions, and thiol. The findings of this study indicated that temulawak extract possesses potent hyaluronidase inhibitory properties. This may be due to the abundance of tannin in temulawak extract. An in vitro study has shown the ability of tannin-rich plants to prevent the release of hyaluronidase from activated neutrophils (Kolakul & Sripanidkulchai 2017).

In this study, temulawak extract demonstrated potent anti-tyrosinase properties at a concentration of 100 µg/mL (82.72%), with an IC_{50} value of 55.87 µg/mL. The IC_{50} value for temulawak extract, which possesses potent anti-tyrosinase properties, has been found to range from 50 to 100 µg/mL, as demonstrated in earlier studies (Tu & Tawata 2015, Dewi et al. 2020). The tyrosinase enzyme stimulates pigmentation processes. This copper-containing enzyme is responsible for catalyzing the synthesis of melanin. L-DOPA, which activates the melanogenic pathway, is the substrate of tyrosinase. Tyrosinase transforms L-DOPA into dopaquinone, which interacts with cysteine to produce melanin, particularly brown melanin. Tyrosinase inhibitors are widely used in skin whitening research to effectively suppress pigmentation processes (Pillaiyar et al. 2017, Varghese et al. 2021). The results of this study revealed that temulawak extract

was identified as a substantial tyrosinase inhibitor. This could be attributed to the structural similarity between the hydroxyl group in the composition of temulawak extract phenolic compounds and the substrate of tyrosinase (L-DOPA) (Uchida et al. 2014). The hydroxyl group of phenolic substances is connected to the effectiveness of tyrosinase inhibitors. This group directly inhibits enzymatic activity by forming hydrogen bonds in the active areas of enzymes, thereby inducing steric hindrance and conformational alterations (Sun et al. 2017).

Strength and limitations

This study provides insight into the anti-aging testing of temulawak extract, including inhibition of elastase, hyaluronidase, and tyrosinase. It found that temulawak extract has strong anti-aging activity, which had not been widely explored in other studies. However, this study had limitations, although an in vitro study is an important first step before an in vivo study. Therefore, further in vivo research is necessary to validate the assertion regarding the anti-aging properties of temulawak.

CONCLUSION

Temulawak extract has anti-aging properties that can effectively inhibit the enzymes elastase, hyaluronidase, and tyrosinase. Thus, temulawak may be applied as a cosmetic ingredient formulated to impede the aging process.

Acknowledgment

The authors express gratitude to Maranatha Christian University, Bandung, Indonesia, for generously offering financial support for this study. Additionally, the authors thank Aretha Medika Utama Biomolecular and Biomedical Research Center, Bandung, Indonesia, for providing research methods and facilities. The helpful support provided by Fadhilah Haifa Zahiroh, Adilah Hafizha Nur Sabrina, Hanna Sari Widya Kusuma, Faradhina Salfa Nindya, Annisa Firdaus Sutendi, and Nindia Salsabila Mia Dewi is also acknowledged.

Conflict of interest

None.

Ethical consideration

The declaration of ethical exemption for this study was issued by the Research Ethics Committee, Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia, with reference No. 001/SRT/KEP/XI/2023 dated 13/11/2023.

Funding disclosure

The research grant for this study was awarded by Maranatha Christian University, Bandung, Indonesia, under contract No. 013/SK/ADD/UKM /III/2023.

Author contribution

TLW and WW contributed to the conception and design, the critical revision of the article for important intellectual content, and the final approval of the article. ASM contributed to the analysis and interpretation of the data, the drafting of the article, statistical expertise, and administrative, technical, or logistic support. RR contributed to the analysis and interpretation of the data, the drafting of the article, and the collection and assembly of the data.

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Original Research Report

MATERNAL MID-UPPER ARM CIRCUMFERENCE AS A SCREENING TOOL TO PREDICT INFANT BIRTH WEIGHT

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ABSTRACT

Mid-upper arm circumference (MUAC) is commonly used for assessing chronic energy deficiency in women of childbearing age. The measurement of MUAC during pregnancy mainly serves as early detection of potential low birth weight. However, certain studies have indicated no significant correlation between maternal MUAC and birth weight. Therefore, this study aimed to determine the relationship between maternal nutritional status and infant birth weight. The study used an analytical observational method with a cross-sectional approach, involving a sample of 86 mothers who delivered at Jagir Primary Healthcare Center in Surabaya, Indonesia, between July and December 2019. The participants were selected based on certain inclusion and exclusion criteria. The data were obtained from secondary sources, specifically the medical records of Jagir Primary Healthcare Center. The data were analyzed using the Spearman test, with a 95% confidence interval and a 5% margin of error. Most mothers (86.05%) had good nutritional status, as indicated by a MUAC measurement of ≥23.5 cm. Only 3.49% of infants were born with a low birth weight (<2,500 g), while 1.16% of infants were considered macrosomia (>4,000 g). Although most mothers exhibiting low MUAC did not give birth to infants with low birth weights, the analysis revealed a significant relationship (p=0.035) between maternal MUAC and infant birth weight. In conclusion, maternal MUAC can be utilized as a screening tool to predict infant birth weight because it indicates the condition of muscle tissue and subcutaneous fat, which serve as the mother's energy reserves. However, several variables can also impact infant birth weight, including maternal nutrient intake.

Keywords: Nutritional status; mid-upper arm circumference; birth weight; malnutrition

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Article history
●Submitted 8/10/2023 ● Revised 6/11/2023 ● Accepted 20/11/2023 ● Published 10/12/2023

How to cite: Fathurrachman MR, Umijati S, Kurniawati EM, et al (2023). Maternal Mid-Upper Arm Circumference as a Screening Tool to Predict Infant Birth Weight. Folia Medica Indonesiana 59 (4), 359-364, https://doi.org/10.20473/fmi.v59i4.50422

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

Highlights:
1. This study investigated the correlation between maternal mid-upper arm circumference and infant birth weight, aiming to present a different outcome compared to previous research.
2. The study's findings offer data suggesting that mid-upper arm circumference can serve as a screening tool for predicting infant birth weight.

INTRODUCTION

Chronic energy deficiency is a prolonged condition in which an individual's nutritional intake fails to adequately meet their body's energy requirements, resulting in adverse effects on their overall health. In adults, chronic energy deficiency leads to detrimental changes in bodily functions, increased risks of morbidity and mortality, and impaired mental and cognitive development, which eventually contribute to decreased productivity. Inadequate food intake, socio-economic status, and infectious diseases are the primary factors that contribute to the occurrence of chronic energy deficiency in adults. Chronic energy deficiency can be assessed by analyzing nutritional status through

the utilization of body mass index (BMI) measures (Tejayanti 2020, Dagne et al. 2021).

Maternal nutritional status can be assessed using MUAC as an alternative to BMI. MUAC measurements are typically carried out on women within the reproductive age range of 15–49 years to assess their nutritional status and identify the presence of chronic energy deficiency (Das et al. 2020, Shifraw et al. 2021). According to the 2018 Basic Health Research, the occurrence of chronic energy deficiency among women of reproductive age in Indonesia was recorded at 14.5% for non-pregnant women and 17.3% for pregnant women. The prevalence rates exhibited a decline in comparison to the rates recorded in 2013, which reached 20.8% for non-pregnant women and 24.2% for pregnant women. However, the prevalence rate of chronic energy deficiency among women aged 15–45 years in 2007 was recorded at 13.6%, a number that was lower than the prevalence observed in 2018 (Minister of Health of the Republic of Indonesia 2013, 2018).

Chronic energy deficiency in pregnant women leads to the delivery of infants with low birth weight. Infants with a low birth weight are susceptible to various disorders, such as stunting. They also have a higher risk of mortality compared to infants with a normal birth weight (Muliawati 2013; Kusumawati et al. 2015). According to data from the World Health Organization (2020), Indonesia ranked 7th in terms of infant mortality. Nevertheless, statistical data demonstrate a consistent downward trend in the infant mortality rate over the years.

The measurement of maternal MUAC has been acknowledged as an effective screening tool for evaluating nutritional status. Furthermore, it has been linked to the weight of newborn infants (Kpewou et al. 2020). However, a previous study conducted by Babu et al. (2021) demonstrated the absence of a relationship between maternal nutrition and infant birth weight. Therefore, this study aimed to ascertain the relationship between the nutritional status of mothers and the birth weight of their infants.

MATERIALS AND METHODS

This study examined the relationship between maternal nutritional status and infant birth weight. Therefore, the research used an analytical observational design with a cross-sectional approach. The data analyzed in this study were acquired from medical records provided by Jagir Primary Healthcare Center, Surabaya, Indonesia. The medical records contained information regarding mothers who delivered their newborns at the health center throughout the period of July to December 2019 (Indriyani et al. 2023).

The subjects were determined using simple random sampling, while the sample size was calculated using Slovin's formula. A total of 86 medical records were obtained from Jagir Primary Healthcare Center. The inclusion criteria for the subjects were mothers aged 15–49 years who had no pregnancy complications or a history of infectious disease. Twin pregnancies were excluded from this study (Minister of Health of the Republic of Indonesia 2018, Isip 2019).

The assessment of maternal nutritional status was conducted by measuring the MUAC. A maternal MUAC measurement below 23.5 cm was deemed indicative of a chronic energy deficit. Infants with a birth weight below 2,500 g were classified as having a low birth weight, whereas those with a birth weight above 4,000 g were defined as having macrosomia (Thamaria et al. 2017, Minister of Health of the Republic of Indonesia 2018).

This study received ethical clearance from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia (No. 161/EC/KEPK/FKUA/2023 dated 26/6/2024). Data obtained from the subjects were processed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, N.Y., USA). The Spearman's correlation test was employed to analyze the correlation between maternal nutritional status and infant birth weight. The correlation was assessed using a 95% confidence interval and a 5% margin of error. A correlation analysis result with a value less than 0.05 ($p<0.05$) signified a significant correlation, whereas a result greater than 0.05 ($p>0.05$) indicated a lack of statistical significance. The correlation coefficient (Rs) was calculated to determine the strength of the relationship between variables. A higher R-value indicated a stronger correlation, while a lower R-value indicated a weaker correlation ($0 \leq R \leq 1$) (Sangi et al. 2021, Barcelona Field Studies Centre 2024).

RESULTS

This study acquired data from the medical records of 86 mothers who delivered their babies at Jagir Primary Healthcare Center over the period of July to December 2019. Table 1 shows the characteristics of the mothers and their infants. The majority of the mothers (86.1%) delivered their babies at the ideal age, with an average age of 27.01 years. In addition, most of the mothers (96.5%) gave birth at full term, with an average gestational age of 39.54 weeks. A total of 13.9% of the mothers were identified as being susceptible to chronic energy deficiency.

According to the data collected from the subjects, there was a higher prevalence of female infants compared to male infants. Only a small number of infants were born with a low birth weight, while the majority were born with an ideal birth weight. The average birth weight of the infants was 3,110.76 g.

Table 1. Characteristics of the mothers and their infants.

Characteristics			n	%	Total
Mothers	Age	<20 years	8	9.3	86 (100%)
		20–35 years	74	86.1	
		>35 years	4	4.6	
	MUAC	<23.5 cm	12	13.9	
		≥23.5 cm	74	86.1	
	GA	<37 weeks	2	2.3	
		37–41 weeks	83	96.5	
		>41 weeks	1	1.2	
Infants	Sex	Male	41	47.7	
		Female	45	52.3	
	BW	<2,500g	3	3.5	
		2,500–4,000g	82	95.3	
		>4,000g	1	1.2	

Notes: MUAC=mid-upper arm circumference; GA=gestational age; BW=birth weight.

Table 2 demonstrates that only three infants (3.5%) had a low birth weight. These infants were not born from mothers at risk of chronic energy deficiency but from mothers with a normal nutritional status instead. Mothers who were at risk of chronic energy deficiency delivered babies with a birth weight within the normal range.

Table 2. Distribution of the maternal mid-upper arm circumference and infant birth weight.

Characteristics		MUAC				Total
		<23.5 cm		≥23.5 cm		
		n	%	n	%	
BW	<2,500g	0	0.0	3	3.5	86 (100%)
	2,500 – 4,000g	12	14.0	70	81.4	
	>4,000g	0	0.0	1	1.1	

Notes: MUAC=mid-upper arm circumference; BW=birth weight.

The statistical analysis using the Spearman's correlation test revealed a significant correlation between maternal nutritional status and infant birth weight, with p=0.035. However, the correlation between maternal MUAC and infant birth weight was found to be weak, with R=0.227 (Table 3).

Table 3. Correlation analysis of maternal mid-upper arm circumference and infant birth weight.

		MUAC	BW
MUAC	Spearman's correlation	1	0.227
	Significance (two-tailed)		0.035
BW	Spearman's correlation	0.227	1
	Significance (two-tailed)	0.035	

Notes: MUAC=mid-upper arm circumference; BW=birth weight.

DISCUSSION

In this study, the majority of the mothers (86.05%) had an MUAC of ≥23.5 cm. Meanwhile, 4.65% of the young mothers had a smaller MUAC. This finding aligns with previous research conducted by Ariendha et al. (2020). The research revealed a higher prevalence of young pregnant women with an MUAC of ≥23.5 cm (79.7%) compared to those with a smaller MUAC (66.67%). The 2018 data on the East Java region of Indonesia showed a high prevalence of pregnant women, including teenage mothers, with an average MUAC of ≥23.5 cm (Minister of Health of the Republic of Indonesia 2018). Nevertheless, female adolescents are more likely to experience chronic energy deficiencies. This is due to their high nutrient requirements for supporting optimal body growth. In addition, female adolescents often limit their food intake to maintain their body weight. Consequently, their MUAC, as represented in numerous studies, becomes smaller than the expected measurements (Retni et al. 2016, Paramata & Sandalayuk 2019).

All of the young mothers in this study delivered their babies at the expected gestational age, which was considered full term. Likewise, the majority of the older mothers gave birth to their babies at full term, while only a small percentage of them (1.16%) delivered their babies after the expected date (post-term). Conversely, those who gave birth at a lower gestational age (pre-term) were mothers of ideal age. In a prior investigation conducted by Zulaikha & Minata (2021), it was discovered that 40 mothers (59.7%) with a risky age delivered their babies prematurely. The study highlighted that a mother of a risky age has a 2.781 times higher likelihood of experiencing preterm birth compared to a mother within the ideal age range. This statement has been supported by several other studies (Wahyuni & Rohani 2017, Drastita et al. 2022).

Out of the total number of babies examined in this study, only three infants (3.49%) were born with a low birth weight, and two of them were female. The

current data is comparable to the 2018 data, which indicated a higher incidence of low body weight in female infants compared to male infants in East Java (Minister of Health of the Republic of Indonesia 2018). A prior study conducted by Iltaf et al. (2017) corroborated this finding. Nevertheless, the sex of the infant is not a definitive determinant of the occurrence of low birth weight. The child's weight during growth is not influenced by sex (Thamaria et al. 2017).

This study found that mothers with a small MUAC did not deliver babies with a low birth weight. They gave birth to babies with a birth weight within the normal range instead. In a recent study conducted by Sangi et al. (2021), it was found that only 26.3% of infants born to mothers with nutritional problems had a low birth weight. However, there is an opposing notion suggesting a correlation between maternal MUAC and infant birth weight. Mothers with a small MUAC were found to have a higher likelihood of giving birth to infants with a low birth weight. Furthermore, mothers with chronic energy deficiency exhibited a fourfold higher risk of low birth weight, even if their MUAC was greater than 22 cm (Kusparlina 2016, Puspitaningrum 2018).

The statistical analysis of this study revealed a significant relationship (p=0.035) between maternal MUAC and infant birth weight, but with a weak correlation coefficient (R=0.227). Similarly, several studies have demonstrated a significant relationship between the two variables, corroborating the results of this study (Rani et al. 2017, Puspitaningrum 2018, Siyoum & Melese 2019). In contrast, a study conducted by Sangi et al. (2021) revealed that there was no statistically significant relationship between maternal MUAC and infant birth weight (p=0.145). However, there was a moderate correlation (R=0.117) observed between the variables. Maternal MUAC indicates the measurement of muscle and subcutaneous fat in the arms, which is one of the areas where fat is stored in the mother's body. However, other factors may affect the birth weight of a baby, including the mother's nutritional intake. While in the womb, the fetus mostly relies on maternal nutrition, particularly for the essential intake of protein and fat. These nutrients are crucial for fetal development and have an immediate impact on the baby's weight (Cohen & Spiegelman 2016, Woldeamanuel et al. 2019).

Strength and limitations

This study offers additional evidence on the advantages of using MUAC measurement as a screening tool to identify the early onset of low birth weight. However, this study primarily relied on MUAC data taken in the first trimester of pregnancy. This imposed a limitation on the quantity of data that could be acquired. Additional research is advised to take into account this factor in order to achieve improved outcomes.

CONCLUSION

Maternal mid-upper arm circumference (MUAC) has an impact on infant birth weight, suggesting its potential as a screening tool for predicting birth weight. Pregnant women must maintain a nutritious diet and meet their nutritional needs to prevent low birth weight. Future research is required to establish the correlation between maternal MUAC and birth weight by examining MUAC measurements immediately after childbirth.

Acknowledgment

The authors would like to thank all the general practitioners, midwives, and staff at Jagir Primary Healthcare Center, Surabaya, Indonesia, especially the Department of Maternal and Child Health.

Conflict of interest

None.

Ethical consideration

The Health Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, issued the ethical approval for this study (No. 161/EC/KEPK/FKUA/2023 dated 26/6/2024).

Funding disclosure

None.

Author contribution

MRF contributed to the conception and design, analysis and interpretation of the data, drafting of the article, and collection and assembly of the data. SU was responsible for the analysis and interpretation of the data, critical revision of the article for important intellectual content, final approval of the article, and statistical expertise. EMK and BS contributed to the critical revision of the article for important intellectual content and final approval of the article.

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Original Research Report

EXPRESSION OF MELANOMA ANTIGEN GENES A11 AND A12 IN NON-SMALL CELL LUNG CANCER

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ABSTRACT

The melanoma antigen gene (MAGE) belongs to the group of cancer-testis antigens that are exclusively expressed in germ cells but may be re-expressed in cancer cells. The highly expressed MAGE-A subfamily in lung cancer may potentially be a diagnostic and prognostic biomarker. This study aimed to identify MAGE-A11 and MAGE-A12 expressions in lung tumors obtained from core biopsy, forceps biopsy, and bronchoalveolar lavage specimens. A cross-sectional observational study was conducted on 90 patients clinically diagnosed with lung tumors. These patients received core biopsy, forceps biopsy, and bronchoalveolar lavage interventions after ethical approval was obtained. The complementary deoxyribonucleic acid (cDNA) quality was assessed by the polymerase chain reaction (PCR) of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The assessment was performed to ascertain if all specimens exhibited positive PCR amplification of the GAPDH gene. MAGE-A11 and MAGE-A12 were identified through a semi-nested reverse transcription PCR. The positive results were detected by measuring the PCR products, with MAGE-A11 and MAGE-A12 at base pairs (bp) of 858 and 496 in the first and second rounds, respectively. The expressions of MAGE-A11 and MAGE-A12 were observed in 3 (3.33%) and 40 (44.44%) out of 90 specimens, respectively. The prevalence rate of non-small cell lung cancer (NSCLC) was 31.11% (28/90). Among these cases, 3.57% (1/28) showed the expression of MAGE-A11, while 32.14% (9/28) exhibited the expression of MAGE-A12. Sixty-two (68.89%) out of 90 patients were diagnosed with no tumor cell malignancy. Out of 62 cases, 2 (3.23%) exhibited the expression of MAGE-A11, while 31 (50%) demonstrated the expression of MAGE-A12. MAGE-A11 and MAGE-A12 were detected in NSCLC and certain specimens with a pathological diagnosis that indicated the absence of malignant cells. In conclusion, MAGE A11 and MAGE A12 have potential markers to improve the pathological diagnosis of lung cancer. Further investigation is necessary to explore the expression of MAGE-A in correlation with lung cancer progression.

Keywords: Cancer; lung cancer; melanoma antigen gene A (MAGE A); mortality; reverse transcription polymerase chain reaction (RT-PCR)

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Article history
●Submitted 10/10/2023 ● Revised 16/11/2023 ● Accepted 20/11/2023 ● Published 10/12/2023

How to cite: Mastutik G, Rahniayu A, Marhana, IA, et al (2023). Expression of Melanoma Antigen Genes A11 and A12 in Non-Small Cell Lung Cancer. Folia Medica Indonesiana 59 (4), 365-371, https://doi.org/10.20473/fmi.v59i4.50477

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

Highlights:
1. In this study, new primers designed using the semi-nested polymerase chain reaction (PCR) method were utilized to identify MAGE-A11 and MAGE-A12 expressions in specimens collected from core biopsy, forcep biopsy, and bronchoalveolar lavage.
2. The histopathological analysis revealed positive expressions of MAGE-A11 and MAGE-A12 in specimens diagnosed with non-small cell lung cancer (NSCLC) as well as in specimens with no malignant cells.
3. This study provides evidence indicating that the detection of messenger ribonucleic acid (mRNA) of MAGE-A11 and MAGE-A12 by nested reverse transcription PCR can improve the accuracy of lung cancer diagnosis.

INTRODUCTION

The melanoma antigen gene (MAGE) is part of the cancer-testis antigens, which are exclusively expressed in germ cells such as the testis, placenta, and ovary. The MAGE family comprises two distinct variations, i.e., type I and type II (Weon & Potts 2015, Õunap et al. 2018). Type I consists of the subfamily members of MAGE-A, MAGE-B, and MAGE-C. The expressions of these MAGE subfamilies are limited to the testis and are rarely found in normal adult cells. Type II includes MAGE-D, MAGE-E, MAGE-F, MAGE-G, MAGE-H, MAGE-L, and necdin genes, which are expressed in normal tissues such as embryonic and adult tissue (Lian et al. 2018).

MAGE-A is a subfamily within type I MAGE. There is a total of 12 subtypes of MAGE-A, specifically MAGE-A1 to MAGE-A12. However, MAGE-A7 is an exception, as it is classified as a pseudogene (Brisam et al. 2016, Mastutik et al. 2021, 2023). MAGE-A is subtly expressed in healthy cells. However, MAGE-A may become reactivated in cancer cells. MAGE-A is highly expressed in various forms of cancer, including thyroid, laryngeal, liver, colorectal, and lung cancers (Lee et al. 2013, Li et al. 2020, Almutairi et al. 2022).

Previous studies have demonstrated that the MAGE-A subfamily is highly expressed in lung malignancies, indicating its potential as a diagnostic and prognostic biomarker of lung cancer. It was found that lung cancer patients who had positive expressions of MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, and MAGE-A6 in their bone marrow had lower survival rates than those who did not exhibit these expressions. Patients with overexpression of all members of the MAGE-A subfamily demonstrated the lowest 10-year survival rate and the worst prognosis (Yi et al. 2017, Sang et al. 2017). The expressions of MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, and MAGE-A6 have been frequently found in patients with distant metastases. It has been found that lymph node metastasis commonly occurs in conjunction with the positive expressions of MAGE-A2, MAGE-A3, MAGE-A4, and MAGE-A6. In addition, the expressions of MAGE-A2, MAGE-A4, and MAGE-A6 have shown a significant association with tumor size changes (Gu et al. 2018). A meta-analysis study revealed that MAGE-A over-expression leads to a low survival rate and unfavorable clinical outcomes. Specifically, increased expressions of MAGE-A1, MAGE-A3, MAGE-A6, MAGE-A9, and MAGE-A10 pose a high risk of mortality and poor clinical outcomes. Furthermore, overexpressions of MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A8, MAGE-A9, MAGE-A10, and MAGE-A12 are associated with a poor prognosis for lung cancer. Therefore, the MAGE-A subfamily has demonstrated its potential as a prognostic indicator for survival in different types of cancer, including lung cancer (Poojary et al. 2020).

MAGE-A11 and MAGE-A12 expressions may have the valuable potential to serve as significant diagnostic and prognostic markers for cancer. MAGE-A11 expression has been associated with advanced stages of tumor and oral cancer, invasion in bladder cancer, and poor overall survival in head and neck cancer cases (Brisam et al. 2016, Jia et al. 2020, Mohsenzadegan et al. 2022). Positive MAGE-A11 expression is commonly observed in breast cancer. The expression has been associated with clinicopathological factors such as estrogen receptor and human epidermal growth factor receptor 2 (HER-2) expression (Hou et al. 2014). Meanwhile, in a study by (Wu et al. 2017), MAGE-A12 over-expression was found to be associated with late-stage gastric cancer, poor prognosis, and a low survival rate of the disease. MAGE-A11 and MAGE-A12 overexpressions were also found to be associated with a poor prognosis and low survival rate in lung cancer. MAGE-A11 and MAGE-A12 may suggest their potential abilities to act as diagnostic and prognostic markers of lung cancer. Therefore, the aim of this study was to identify the MAGE-A11 and MAGE-A12 expressions in lung tumors obtained from core biopsies, forceps biopsies, and bronchoalveolar lavage specimens.

MATERIALS AND METHODS

This cross-sectional observational study was conducted at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The Health Research Ethics Committee of the hospital issued the ethical approval for this study, with reference No. 497/Panke.KKE/VIII/2017 on 25/8/2017. The samples were obtained via core biopsies, forceps biopsies, and bronchoalveolar lavage. This study involved 90 patients with a diagnosis of lung tumors between August 2017 and August 2018. The inclusion criteria were those aged 20–80 years who had a measurable tumor or lesion, underwent a certain intervention (i.e., a core biopsy, a forceps biopsy, or bronchoalveolar lavage), had not received any therapy, and provided informed consent as proof of their voluntary participation in this study. Patients who had a primary tumor in other organs other than the lungs, were unwilling to participate, and had arrhythmia, hypoxemia, hypercapnia, or unstable hemodynamics were excluded from this study (Keung et al. 2020).

Ribonucleic acid (RNA) was extracted according to the instructions of the RNeasy Plus Mini Kit

(Qiagen, batch no. 74134, Hilden, Germany). Afterward, reverse transcription (RT) was performed using the RT-qPCR Master Mix (Toyobo, batch no. FSQ-301, Osaka, Japan) in accordance with the protocols outlined in our previous study (Mastutik et al. 2021). The quality of complementary deoxyribonucleic acid (cDNA) was assessed through a polymerase chain reaction (PCR) for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. The GAPDH primer and PCR protocol were set in a consistent manner with our earlier study (Mastutik et al. 2021). Once all specimens were confirmed as positive for the GAPDH gene, PCR was then performed to identify MAGE-A11 and MAGE-A12.

The semi-nested reverse transcription polymerase chain reaction (RT-PCR) was performed to identify MAGE-A11 and MAGE-A12. The forward and reverse primers used for the identification of MAGE-A11 in the first round were MF11: 5'-GGA GGA GAA CAA GTG CTG TGG-3' and MR11: 5'-CAC CAG GTA CTT TTC CTG CAC-3', respectively. In the second round, the forward and reverse primers for the identification of MAGE-A11 were MF11 and MR12: 5'-CCA GYA TTT CTG CCT TTG TGA-3', respectively. Additionally, the forward and reverse primers used for the identification of MAGE-A12 in the first round were MF12: 5'-CCA AGC ATC CAG GTT CTG AGG-3' and MR10: 5'-CTC CAG GTA STT YTC CTG CAC-3', respectively. Meanwhile, the forward and reverse primers used for the identification of MAGE-A12 in the second round were MF12 and MR12. The PCR protocol was implemented following the same method as outlined in our previous study on the MAGE-A family (Mastutik et al. 2021). MAGE-A11 and MAGE-A12 were identified by assessing the positive results, which were indicated by PCR products of 858 base pairs (bp) and 496 bp for the first round and second round, respectively. After the data were analyzed, they were tabulated and presented in figures and tables.

RESULTS

This study was conducted on 90 patients with a clinical diagnosis of lung tumors. The patients underwent core biopsy, forceps biopsy, or bronchoalveolar lavage procedures. The youngest patient was 21 years old, while the oldest was 79 years old. Most of the patients fell within the age group of 51–50 years old, with the following highest proportions in the age groups of 41–50 years and 61–70 years. A total of 60 (66.67%) out of 90 patients were male. The pathological diagnosis showed that 28 (31.11%) out of 90 patients had non-small cell lung cancer (NSCLC) types, including adenocarcinoma and squamous cell carcinoma. A majority of the specimens, specifically 62 (68.89%) out of 90, did not exhibit any presence of malignant cells. This was particularly evident in the specimens obtained from bronchoalveolar lavage, as shown in Table 1.

Out of the 90 specimens examined, MAGE-A11 expression was detected in 3 specimens (3.33%), yet MAGE-A12 expression was observed in 40 specimens (44.44%) (Table 2). Out of the 28 samples diagnosed with NSCLC, 1 sample (3.57%) displayed MAGE-A11 expression, and 9 samples (32.14%) demonstrated MAGE-A12 expression. In addition, among the 62 samples that did not contain malignant cells according to the pathological analysis, 2 (3.23%) demonstrated MAGE-A11 expression, and 31 (50%) exhibited MAGE-A12 expression (Table 3).

Table 1. Characteristics of the patients diagnosed with lung tumors.

Variables		n	%
Age (years)			
21-30		5	5.55
31-40		4	4.44
41-50		23	25.55
51-60		34	37.78
61-70		18	20
71-79		5	5.55
Mean±SD:	53.82±11.34		
Min-max:	21-79		
Sex		60	66.67
Male		30	33.33
Female			
Specimens		31	34.44
Core biopsy		19	21.11
Forceps biopsy		40	44.44
BAL			
Histopathological diagnosis			
NSCLC, adenocarcinoma		25	27.78
NSCLC, squamous cell carcinoma		3	3.33
No cancer cells found		62	68.89

Notes: SD=standard deviation; NSCLC=non-small cell lung cancer; BAL=bronchoalveolar lavage.

Table 2. Distribution of MAGE-A11 and MAGE-A12 expressions.

Gene expression	Positive		Negative	
	n	%	n	%
MAGE-A11	3	3.33	87	96.67
MAGE-A12	40	44.44	50	55.55

Table 3. Distribution of MAGE-A11 and MAGE-A12 expressions according to the pathological diagnosis.

MAGE-A Expression	NSCLC		No malignant cells found	
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
MAGE-A11	1 (3.57)	27 (96.43)	2 (3.23)	60 (96.77)
MAGE-A12	9 (32.14)	19 (67.86)	31 (50)	31 (50)

Note: NSCLC=non-small cell lung cancer.

DISCUSSION

Lung cancer is the most common form of cancer and has a high mortality rate worldwide. The 2020 data from the Global Cancer Observatory (GLOBOCAN) revealed that the estimated mortality rate for lung cancer was 1.8 million. In the United States, lung cancer ranks as the second most prevalent cancer and causes the highest number of deaths in both men and women (Sung et al. 2021, Siegel et al. 2023). In Indonesia, the disease is the third most common cancer after breast and uterine cervical cancers. There were 34,783 new cases, accounting for 8.8% of all cancer cases. Lung cancer is the most common type of cancer in men, with 25,943 new cases (14.1%). Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the most prevalent histopathological types of lung cancer (Gu et al. 2018). Lung cancer is frequently diagnosed after the disease has progressed to an advanced stage. This is because of the unclear symptoms presented during the early stages of the disease, leading to a high mortality rate (Cainap et al. 2020). Symptoms that appear once the cancer has progressed to an advanced stage typically result in a poor prognosis for patients. Moreover, the five-year survival rate is around 16%. Meanwhile, the approximate five-year survival rate for patients with stage IV lung cancer is below 10% (Ning et al. 2021). Therefore, it is important to carry out screening and be aware of the early symptoms of cancer in individuals with major risk factors for lung cancer, such as tobacco smoking. Appropriate methods for collecting specimens in diagnostics and identifying certain biomarkers have attracted the interest of experts to improve diagnosis accuracy (Malhotra et al. 2016).

This study investigated specific biomarkers, i.e., MAGE-A11 and MAGE-A12, by using several methods to collect specimens through core biopsy, forceps biopsy, and bronchoalveolar lavage procedures. Specimens from the periphery of the chest cavity were obtained by core biopsies using ultrasonography or computed tomography (CT) guidance, while specimens from the central chest cavity were acquired by forceps biopsies and bronchoalveolar lavage. The aforementioned methods are considerably safe and feasible to use in collecting specimens for pathological or molecular diagnosis (Marhana et al. 2022). However, these methods are quite invasive and can result in difficulties, such as too few specimens or insufficient exfoliated cells for pathological diagnosis. In addition, there is a potential increase in the risk of bleeding (Goel et al. 2022). Hence, the use of a polymerase chain reaction to detect MAGE-A11 and MAGE-A12 may support pathological examination in molecular diagnosis.

This study identified MAGE-A11 expression in 3 (3.33%) out of 90 specimens, while MAGE-A12 expression was detected in 40 (44.44%) out of 90 specimens. According to the pathological diagnosis, 28 patients were found to have NSCLC, while 62 patients showed no presence of malignant cells on the slides. It is interesting that the investigation in this study could detect MAGE-A11 and MAGE-A12 expressions in specimens that were pathologically determined to be devoid of malignant cells. The results showed that 2 (3.23%) out of 62 specimens were positive for MAGE-A11 expression, while 31 (50%) out of 62 specimens exhibited positive MAGE-A12 expression. Pathological examination is considered the gold standard for diagnosing malignancy. Accurate diagnosis is crucial for the patient because the selection of the appropriate treatment, such as targeted therapy, is contingent on the type of cancer (Ning et al. 2021). Pathological diagnosis relies on the examination of cell morphology, necessitating a sufficient number of observable cells on the slide. However, cancer tissue is fragile, and its cells are easily lysed, making them prone to damage and destruction during the bronchoscopy process. This can cause the cells to be undetectable on the pathology slide. This study showed that the expressions of MAGE-A11 and MAGE-A12 were detected even in specimens that were pathologically defined as devoid of cancer cells. Therefore, the utilization of semi-nested RT-PCR to detect MAGE-A11 and MAGE-A12 can serve as an additional method for identifying cancer cells from core biopsy, forceps biopsy, and bronchoalveolar lavage specimens.

The MAGE-A subfamily genes, including MAGE-A11 and MAGE-A12, are silenced in normal cells

by the process of deoxyribonucleic acid (DNA) methylation. However, MAGE-A11 and MAGE-A12 have been known to undergo reactivation in cancer cells, particularly in the early stages of carcinogenesis, due to epigenetic changes such as demethylation or histone acetylation. The overexpression of MAGE-A12 promotes the degradation of p21, a tumor suppressor gene (Zhao et al. 2019). In normal cells, p21 regulates cell cycle arrest together with retinoblastoma protein (pRB). The activation of p53 leads to an increase in p21 expression, which in turn causes the formation of the retinoblastoma-E2F transcription factor (RB-E2F) complex. This complex then downregulates a number of genes related to the cell cycle, resulting in cell cycle arrest (Engeland 2022). The MAGE-A12 gene promotes cell cycle progression, immortality, and anti-apoptosis in cancer cells by causing the degradation of p21. In addition, the increased expression of MAGE-A11 was associated with DNA hypomethylation in the promotor region. In other conditions, the increased MAGE-A11 expression was found to be related to both DNA hypomethylation and histone acetylation (James et al. 2013, Yanagi et al. 2017). In their study, Su et al. (2013) reported that MAGE-A11 promotes carcinogenesis by specifically affecting the retinoblastoma (RB) pathway. The study additionally showed that MAGE-A11 interacts with the p107-RB-related protein. MAGE-A11 was found to be associated with retinoblastoma-like 1 (p107), resulting in the stabilization of p107. This interaction can inhibit the ubiquitination or degradation of p107 and result in the hypophosphorylation of E2F transcription factor 1 (E2F1). The hypophosphorylation of E2F1 causes the stabilization and activation of E2F1, which subsequently leads to cell cycle progression and anti-apoptosis.

MAGE-A11 and MAGE-A12 expressions are upregulated in several types of cancer. MAGE-A11 was identified in cases of squamous cell carcinoma in both head and neck cancer and lung cancer. An elevated expression of MAGE-A11 has been related to poor overall survival. The study conducted by Jia et al. (2020) revealed that MAGE-A11 expression was higher in cancer tissue than in the surrounding tissue. Moreover, this increased expression was found to be associated with the occurrence of lymph node metastasis. It was found that MAGE-A11 and MAGE-A12 are associated with the progression of oral cancer to advanced stages. Furthermore, increased MAGE-A11 expression was found to be associated with invasive and advanced-stage bladder cancer. The progression of gastric cancer to advanced stages was also found to be related to increased MAGE-11 expression (Wu et al. 2017, Mohsenzadegan et al. 2022). The study conducted by Sang et al. (2017) used tissue microarray immunohistochemistry to demonstrate that the overexpression of MAGE-A11 and MAGE-A12 was associated with the lowest ten-year survival rates in patients with lung adenocarcinoma, indicating a poor prognosis. The expression of MAGE-A, specifically MAGE-A11 and MAGE-A12, has been associated with the lowest survival rate in lung cancer patients. MAGE-A11 and MAGE-A12 may potentially serve as prognostic markers in certain types of cancer, such as lung cancer. Additionally, it has been proposed that MAGE-A12 may be useful as an additional marker for early detection of oral cancer (Brisam et al. 2016, Poojary et al. 2020). Hence, in cases where cancer cells are absent but MAGE-A11 or MAGE-A12 expressions are detected, it is necessary to obtain new specimens for a reevaluation of the histopathological diagnosis. This is due to the potential failure of the specimen collection or lysis, resulting in the absence of cancer cells during the initial diagnosis.

Strength and limitations

This study detected the presence of MAGE-A11 and MAGE-A12 expressions in small tissue samples obtained from core biopsies and forceps biopsies, as well as in fluid obtained from bronchoalveolar lavage during bronchoscopy. MAGE-A11 and MAGE-A12 expressions were observed in specimens diagnosed with NSCLC as well as in specimens without any malignant cells. Thus, this study suggests that using nested RT-PCR to detect the mRNA of MAGE-A11 and MAGE-A12 can improve the accuracy of lung cancer diagnosis. However, this study has limitations as it solely evaluated MAGE-A11 and MAGE-A12 expressions in lung tumors obtained from core biopsy, forceps biopsy, and bronchoalveolar lavage procedures without establishing any relationship with the stage and invasion of the malignancies. Further investigation is required to examine the relationship between MAGE-A11 and MAGE-A12 expressions and the progression and prognosis of lung cancer.

CONCLUSION

MAGE-A11 and MAGE-A12 expressions are detectable in non-small lung cancer (NSCLC). These expressions can be identified in specimens that have been pathologically diagnosed with NSCLC, as well as in those that do not contain malignant cells. Therefore, MAGE-A11 and MAGE-A12 have potential as markers that can support the pathological diagnosis of lung cancer.

Acknowledgment

We would like to thank the Ministry of Research,

Technology, and Higher Education of the Republic of Indonesia for their support in funding this research. We would also like to express our gratitude to Universitas Airlangga, Surabaya, Indonesia, the patients who participated in this study, and the pulmonologists (Doctor CDL, Doctor MS, and Doctor MM) who assisted in collecting specimens for this study.

Conflict of interest

None.

Ethical consideration

This study received ethical approval from the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, with reference No. 497/Panke.KKE/VIII/2017 on 25/8/2017.

Funding disclosure

The Ministry of Research, Technology, and Higher Education of the Republic of Indonesia provided funding for this study through the Higher Education Excellent Basic Research Program, with contracts No. 200/UN3.14/LT/2018 and 533/UN3.14/LT/2019.

Author contribution

GM contributed to the conception and design, the analysis and interpretation of the data, the drafting of the article, critical revision of the article for important intellectual content, final approval of the article, and the acquisition of funding. AR contributed to the analysis and interpretation of the data, the drafting of the article, the critical revision of the article for important intellectual content, and the final approval of the article. IAM contributed to the final approval of the article, the provision of study materials or patients, and the collection and assembly of data. MA contributed to the analysis and interpretation of the data, the final approval of the article, and the provision of administrative, technical, or logistic support. HFT contributed to the final approval of the article, the provision of administrative, technical, or logistic support, and the collection and assembly of data. RI contributed to the analysis and interpretation of the data, the final approval of the article, and the collection and assembly of the data.

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Original Research Report

DIFFERENCES IN THE DEVELOPMENT OF LANGUAGE AND SOCIAL INDEPENDENCE IN CHILDREN WITH SPEECH DELAY AND SENSORINEURAL HEARING LOSS BASED ON THE AGE FOR EARLY INTERVENTION AND THE DURATION OF AUDITORY-VERBAL THERAPY

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ABSTRACT

Speech delay is primarily attributed to sensorineural hearing loss, which may significantly impact a child's language development and social independence. Consistent and periodic auditory-verbal therapy as an early intervention has the potential to positively influence language development, thereby fostering independence in children. The objective of the research was to examine the differences in the development of language and social independence among children who have speech delay and sensorineural hearing loss at the children's age for early intervention and with the duration of auditory-verbal therapy taken into consideration. The research employed an analytical-observational design with a cross-sectional approach and total sampling. The sample comprised 29 children who were undergoing the weekly routine of auditory-verbal therapy at Yayasan Aurica, Surabaya, Indonesia. The tool utilized in this study was the Pre-Screening Developmental Questionnaire. The data underwent bivariate analysis, specifically using the Chi-squared test with a significance level set at a p-value of <0.05. The language development analysis resulted in a p-value of 0.013 for the age variable and a p-value of 0.019 for the therapy duration variable. Meanwhile, the social independence analysis yielded a p-value of 0.229 for the age variable and a p-value of 0.111 for the therapy duration variable. In conclusion, the influence of age on early intervention had a significant difference from that of the duration of auditory-verbal therapy on the language development of children with speech delay and sensorineural hearing loss. Conversely, age and therapy duration did not exert any meaningful difference in terms of their influence on the children's social independence development.

Keywords: Sensorineural hearing loss; auditory-verbal therapy (AVT); inclusive health; early intervention

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Article history
●Submitted 13/10/2023 ● Revised 11/11/2023 ● Accepted 1/12/2023 ● Published 10/12/2023

How to cite: Purwanti ZI, Romdhoni AC, Suryawan A (2023). Differences in the Development of Language and Social Independence in Children with Speech Delay and Sensorineural Hearing Loss based on the Age for Early Intervention and the Duration of Auditory-Verbal Therapy. Folia Medica Indonesiana 59 (4), 372-380, https://doi.org/10.20473/fmi.v59i4.51495

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

Highlights:
1. This study explored the significance of auditory-verbal therapy as an early intervention for children with speech delay and sensorineural hearing loss, a topic that deserves further research in developing countries such as Indonesia.
2. The findings highlight the significance of age and therapy duration on the language development of children with speech delay and sensorineural hearing loss.

INTRODUCTION

Speech delay refers to a condition where a child's ability to speak develops slower in comparison to other children of the same age. The cause of this delay is frequently attributed to the presence of sensorineural hearing loss. Children who have both speech delay and sensorineural hearing loss

experience language development that significantly falls behind the developmental milestones expected for their age. Furthermore, children with this condition exhibit speech and language development that differs qualitatively from that of typical children (Hartanto 2018). The World Health Organization estimated that there are 466 million individuals worldwide experiencing hearing loss, with 34 million of them being children. Approximately 60% of these cases are preventable (Olusanya et al. 2019). Indonesia ranks fourth among Asian countries due to the high prevalence of hearing loss at 4.6%. Additionally, the prevalence of deafness across all age groups in seven provinces of Indonesia is 0.4%. Globally, it is estimated that 1–3 babies per 1,000 live births are affected by congenital deafness. This rate increases to 2–4 out of every 100 babies in intensive care units (Minister of Health of the Republic of Indonesia 2022).

Maulana (2021) conducted an investigation on children who were diagnosed with congenital hearing loss at an early stage of their lives. The study revealed that early intervention resulted in an improved quality of life in later years. On the other hand, children who did not receive early intervention exhibited a contrary effect. Children with hearing loss who are not identified early may miss a critical window for language development. This can lead to potential delays and deficiencies in speech and language skills, ultimately limiting their academic achievement and career prospects. The failure to promptly detect and intervene in cases of hearing loss can result in a significant social burden on the affected children, highlighting the need for tailored therapy and education (Purnami et al. 2018). Hearing loss can also impede children's development of social independence, as language plays a crucial role in achieving independence. Children who are unable to effectively communicate their needs and wants, or who struggle to express themselves and are not understood by others, may encounter barriers to developing confidence and independence (Hurlock & Sijabat 2017).

Early intervention is essential for addressing speech delays and hearing loss in children. A common occurrence in society is that parents often become aware of a disorder only when their child exhibits a speech delay. This is largely because they lack awareness regarding the critical importance of early detection and intervention for hearing problems in children (Maulana 2021). The American Academy of Pediatrics Committee and the Joint Committee on Infant Hearing recommend that congenital hearing loss be diagnosed before the age of 3 months and treated before the age of 6 months. This pertains to the critical phase of language development, which starts in the first 6 months of life and continues until the age of 2 years (American Academy of Audiology 2019).

It has been recognized that the critical developmental periods are significant for gaining language skills. Therefore, it is imperative to offer early intervention for children with hearing loss in the domain of language acquisition (Rahardja et al. 2010). Auditory-verbal therapy (AVT) maximizes residual hearing by utilizing hearing aids or cochlear implants to identify sounds. The adoption of contemporary hearing technology has enabled children with hearing impairments or those who are hard of hearing to attain the highest possible acoustic neurological advantages. The primary goal of the auditory-based verbal approach is to empower children to harness their auditory potential for effective communication through spoken language (Putri & Purbaningrum 2020). Parents, therapists, and children engage in playful activities with the goal of facilitating auditory-verbal learning. These activities are intended to enhance the child's residual hearing, allowing them to achieve auditory skills comparable to those of children with typical hearing (Ratih & Rini 2015). Hence, this study aimed to examine the differences in the development of language and social independence among children with speech delay and sensorineural hearing loss. This was achieved by assessing their enrollment age for the early intervention program and the duration of therapy they received.

MATERIALS AND METHODS

This study used an observational analytical design with a cross-sectional approach to investigate the differences in the development of language and social independence among children with speech delay and sensorineural hearing loss. The variables examined included the age at which the children were enrolled in the early intervention program and the duration of auditory-verbal therapy they attended. The cross-sectional design was employed to analyze the relationships between variables and their outcomes by collecting data at a single point in time (Abduh et al. 2022). The inclusion criteria for this research comprised children who underwent regular weekly auditory-verbal therapy and parents who consented to have their children participate as respondents. A total sampling method was employed to select the sample, which consisted of 29 children. The research was conducted at Yayasan Aurica, Surabaya, Indonesia, from February to September 2022, following the approval of the research proposal.

The data collection instrument employed in this research was the Pre-Screening Developmental Questionnaire, which was designed to assess

children's development of language and social independence. The questionnaire was distributed to the parents of the respondents following the completion of the auditory-verbal therapy. The children's development in language and social independence was categorized as normal if all responses on the questionnaire were "yes." Conversely, it was deemed to be at risk for a disorder if there was at least one "no" response on the questionnaire. Additionally, the assessment results were categorized using secondary data pertaining to the age of the children when they started the early intervention program and the duration of therapy they received. The data processing techniques used were editing, coding, data entry, data cleaning, and tabulation methods (Misbahuddin 2022). In this research, a bivariate analysis was performed using the Chi-squared test, with a significance level established at $p<0.05$. The data were further analyzed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) to ascertain differences between the two dependent variables and the two independent variables.

RESULTS

The results of the analysis using the Chi-squared test were organized and presented in the form of frequency distributions. Table 1 summarizes the distribution of respondents according to the degree of their hearing and the assistive devices they used.

Table 1. Distribution of hearing loss and assistive devices among the respondents.

Characteristics	n	%
Hearing thresholds		
0–15 dB	0	0
16–25 dB	0	0
26–40 dB	0	0
41–55 dB	0	0
56–70 dB	2	6.9
71–90 dB	4	13.8
>90 dB	23	79.3
Total	29	100
Devices		
Hearing aid	10	34.5
Cochlear implant	19	65.5
Total	29	100

The results of this study revealed that the majority of the respondents (79.3%) experienced profound hearing loss, as indicated by a threshold above 90 dB. Severe and moderately severe hearing loss were the second and third most prevalent degrees of hearing loss, respectively. Cochlear implants were the most commonly used assistive devices (65.5%) to support the children's remaining hearing. Meanwhile, 34.5% of the respondents used regular hearing aids.

Table 2. Distribution of the respondents' development of language and social independence.

Variables	n	%
Language development		
At risk of disorder	7	24.1
Normal	22	75.9
Total	29	100
Social independence development		
At risk of disorder	11	37.9
Normal	18	62.1
Total	29	100

Table 3. Distribution of the respondents' age for early intervention and the duration of therapy.

Variables	n	%
Age		
≤3 years	16	55.2
>3 years	13	44.8
Total	29	100
AVT duration		
≤2 years	22	75.9
>2 years	7	24.1
Total	29	100

Note: AVT=auditory-verbal therapy.

As displayed in Table 2, 24.1% of the children were identified as being at risk of experiencing impaired language development. Additionally, Table 3 shows that 37.9% of the children were at risk of impaired development of social independence. More than half of the children (55.2%) received early intervention when they were still in the age category of ≤3 years. A majority of the children (75.9%) attended auditory-verbal therapy for a duration of two years or less. These results were additionally analyzed to identify any differences in the development of language and social independence among the respondents.

Table 4. Differences in language development according to age for early intervention and therapy duration.

Variables	Language development				Total		
	At risk of disorder		Normal				
	n	%	n	%	n	%	
Age							
≤3 years	1	6.3	15	93.7	16	100	p=0.013
>3 years	6	46.2	7	53.8	13	100	
Total	7	24.1	22	75.9	29	100	
AVT duration							
≤2 years	3	13.6	19	86.4	22	100	p=0.019
>2 years	4	57.1	3	42.9	7	100	
Total	7	24.1	22	75.9	29	100	

Table 4 presents data on the children's language development according to their age of enrollment in the early intervention program and the duration of therapy they completed. A single respondent (6.3%) who received early intervention at the age of ≤3 years was found to be at risk of disorder. As many

as 46.2% of the respondents who initiated their therapy after the age of 3 years also exhibited a risk of disorder. When examining the duration of auditory-verbal therapy, 13.6% of the children who had therapy for two years or less were found to be at risk of developing a disorder. Among those who underwent therapy for over two years, the proportion of respondents at risk of disorder was 57.1%. Nevertheless, the Chi-squared test revealed significant differences in the respondents' language development according to their starting age (p=0.013) and therapy duration (p=0.019).

Table 5. Differences in the development of social independence according to age for early intervention and therapy duration.

Variables	Social independence development				Total		
	At risk of disorder		Normal				
	n	%	n	%	n	%	
Age							
≤3 years	4	31.8	12	68.2	16	100	p=0.111
>3 years	7	57.1	6	42.9	13	100	
Total	11	37.9	18	62.1	29	100	
AVT duration							
≤2 years	7	31.8	15	68.2	22	100	p=0.229
>2 years	4	57.1	3	42.9	7	100	
Total	11	37.9	18	62.1	29	100	

The data shown in Table 5 demonstrate the respondents' development of social independence in relation to their starting age and therapy duration. As many as 31.8% of the respondents who began receiving early intervention at or before the age of 3 were found to be at risk of developing a disorder. In the meantime, among those who started their therapy after the age of 3, the prevalence of the risk of disorder reached 57.1%. When considering the auditory-verbal therapy, it was found that 31.8% of the children who had received it for a duration of two years or less were at risk of disorder. As many as 57.1% of the respondents who had received therapy for over two years also faced a risk of disorder. Overall, the data indicated no significant differences in the development of social independence according to the age for early intervention (p=0.111) and the duration of therapy (p=0.229).

DISCUSSION

In order to foster language skills, particularly in listening, humans require the sense of hearing as a means to receive information. The development of a child's auditory system in the early stages of life heavily relies on stimulation from the environment, which provides crucial auditory input (Putri & Purbaningrum 2020). According to Wagino & Rafikayati (2013), to minimize the impact of hearing loss, the primary focus should be on the development of language skills. Language development should take precedence over other aspects of development due to its essential nature and role as the primary prerequisite for child development. Proficient language skills in children can contribute to enhanced intellectual development and facilitate their ability to socialize with the environment, thereby fostering the development of social and emotional competencies. Additionally, this can also affect children's independence, as language plays a crucial role in fostering their social development (Hurlock & Sijabat 2017).

Effective management of speech delay necessitates strong collaboration between speech therapists and the rehabilitation team at the children's homes. Hence, parental involvement significantly impacts the success of addressing speech delays in children (Fitriyani et al. 2019, Sholehen et al. 2020). One of the intervention therapies that can be useful is auditory-verbal therapy. Heriyanti (2020) suggested that auditory-verbal therapy is a combined application of techniques, strategies, conditions, and procedures that promote the optimal acquisition of spoken language through listening. This method plays a major role in nurturing deaf children's personal, social, and academic lives. Auditory-verbal therapy is an approach that utilizes auditory stimulation to take advantage of residual hearing (Ratih & Rini 2015). Auditory-verbal therapy also seeks to empower children with hearing loss to effectively engage in verbal communication (both speaking and listening) and to facilitate their growth and learning within broader school and community settings. The purpose of this method is to enable the children to achieve their desired goals and participate more actively in their surroundings.

The analysis of the relationship between the children's language development and their starting age for early intervention showed a significant difference. Among those who were at risk of language impairment, the prevalence of respondents who initiated therapy at an earlier age was higher compared to those who started at a slightly older age. This aligns with the findings of a study by Jauhari (2020), who found that proper early detection and rehabilitation improve children's speech and language development. Delays in conducting timely detection will lead to delays in commencing interventions, which can have a detrimental impact on the children's future growth. According to Hartanto (2018), the first 36 months of life represent a critical period for language development. The rate of language development during this period cannot be replicated at any other stage of life. Children with hearing loss who start therapy late are at a higher risk of permanent speech and language disorders

compared to their peers who receive early intervention.

As Putri & Purbaningrum (2020) suggested, children with hearing loss who have early access to sound amplification and consistent communication exhibit superior spoken language than those who lack such access. Children's language skills continue to develop during a critical period within the first six years of their lives. Beyond this critical phase, their language skills will gradually decline, and the brain will no longer be capable of making significant changes in neural connectivity (American Academy of Audiology 2019). Heriyanti (2020) also corroborated this notion by discussing the ideal age for initiating the use of hearing technology. Early exposure to auditory stimulation through the use of hearing technology enhances language skills comparable to those of hearing individuals. The outcomes achieved will vary between children who use hearing technology from an early age and those who start at a later age.

Early detection and auditory stimulation are of the utmost importance for hearing loss in children. Timely identification of hearing loss in newborns through screening is necessary for immediate treatment (Wagino & Rafikayati 2013, Warasanti et al. 2020). In addition, the early intervention must take into account the critical period for neurological and language development, the assessment of the degree of hearing loss, as well as the utilization of amplification, medical technology, and auditory stimulation. It is crucial to diagnose auditory problems, as well as provide intervention and education, before a child reaches 6 months of age. This is because the auditory brain develops optimally during this period (American Academy of Audiology 2019).

Children who continue to be vulnerable to disorder despite early intervention may potentially be impacted by other contributing factors. Hartanto (2018) proposed that speech and language delays may arise as a consequence of secondary factors linked to various conditions, such as intellectual disabilities, autism spectrum disorders, cognitive impairments, physical disorders, mutism, psychosocial disorders, and bilingualism. Children first acquire language within the boundaries of their familial environment. They initiate the process of hearing and familiarizing themselves with their family's primary language, eventually acquiring the ability to speak the language (Alfin & Pangastuti 2020). Hence, the family serves as an external factor that significantly influences children, playing a pivotal role in shaping their language acquisition process. The approach in which the family encourages and nurtures language skills, especially during the period of growth and development, is of paramount significance. Insufficient stimulation from the family can impede children's language development, resulting in delayed linguistic abilities and impaired communication skills (Khoiriyah et al. 2016, Hurlock & Sijabat 2017).

This study found that there was a significant difference in the children's language development with regards to therapy duration. However, respondents who had a longer therapy duration exhibited a higher prevalence of being at risk compared to those who underwent therapy for a shorter duration. This observation underscores the importance of assessing the outcomes of auditory-verbal therapy not solely based on its duration but rather on its individualized effectiveness for each child (Heriyanti 2020). According to Jauhari (2020), the purpose of auditory-verbal therapy is to reduce children's dependence on lip reading and sign language. When children are diagnosed and treated late, it becomes challenging to ascertain the effectiveness of auditory-verbal therapy. Continuous monitoring is essential to evaluate whether the therapy is efficient in helping the participant attain language skills that are appropriate for their age.

Early diagnosis and active participation of family members can influence the outcomes of auditory-verbal therapy. However, the therapy outcomes can also be affected by other factors, including audiological management, the underlying causes of hearing loss, the degree of hearing impairment, the effectiveness of assistive devices, the emotional well-being of the family, and the competence of the therapist (Heriyanti 2020, Putri & Purbaningrum 2020). Furthermore, there are internal factors that have a significant impact, such as auditory potential, cognitive capacities, developmental stage, individual traits, learning preferences, and overall health. These factors pose a greater impact on the auditory-verbal therapy process, subsequently influencing the attainment of language skills in children (Wagino & Rafikayati 2013).

The active participation of the family is crucial, whereas a lack thereof may profoundly impact the outcomes of auditory-verbal therapy. The effectiveness of auditory-verbal treatment may be compromised if the family has not fully accepted their children's condition, as the emotional well-being of the family is crucial. Furthermore, various levels of intellectual abilities may lead to the children's different aptitudes to absorb all information and instruction provided in auditory-verbal therapy (Estabrooks et al. 2016). If parents fail to comprehend their children's learning style and the challenges, it can impact the effectiveness of auditory-verbal therapy. Therefore, parents must grasp how to create an enjoyable learning

environment for their children, which can enhance their receptiveness to the auditory-verbal therapy lessons (Putri & Purbaningrum 2020).

The age factor remains a significant aspect to consider when assessing the effectiveness of auditory-verbal therapy. This is due to the fact that intervention, such as hearing rehabilitation, has been proven to yield outcomes that are correlated with the age of the children (Aval et al. 2020). The advantages of the therapy may manifest as enhanced emotional well-being, social development, and language and speech skills. Empirical evidence has shown that auditory-verbal therapy yields favorable results even when applied to children older than 3 years. The speech and language abilities of the older children with hearing impairments can be comparable to those of typical-hearing children (Kaipa & Danser 2016, Baungaard et al. 2019).

This study did not find any significant differences in the development of social independence in relation to the children's age for early intervention ($p=0.111$) and therapy duration ($p=0.229$). This contradicts the findings of a study conducted by Hurlock & Sijabat (2017), who suggested that children with speech delays often struggle to achieve their desired independence. This difficulty arises from their limited verbal communication skills, which lead them to be perceived as too young or incapable of doing things independently. This can result in a continuous need for assistance from adults. Such dependency inhibits children from gaining self-confidence and developing independence. Early intervention and consistent therapy, on the other hand, can improve language development and enable children to better express their needs and wants (Hartanto 2018). According to the results of this study, the children's age for early intervention did not exhibit a significant difference in the development of social independence. This implies that other factors exert a more substantial influence on the development of children's social independence.

Prior studies conducted by Sa'diyah (2017) and Setyaningsih & Wahyuni (2018) revealed that the social independence of children is influenced by various factors, including parenting, gender, intelligence, environmental conditions, as well as socioeconomic and sociocultural factors. The process of developing social independence in children begins within the context of familial relationships, primarily with parents or caregivers at home. Children start to interact socially by engaging in play with others, mainly within the family circle. They instinctively begin to acquire the skills necessary for interacting with individuals beyond themselves, particularly those in their immediate surroundings. This process of social interaction gradually extends beyond the confines of the family home. Eventually, this progression extends to school environments, fostering the development of social independence.

As noted by Komala (2015), social independence in children is influenced by their familial upbringing, resulting in differences in independence levels between one child and another. The presence of other individuals in the vicinity of children can either facilitate greater independence or have the opposite effect. The home serves as the primary and fundamental educational environment, consequently placing the duty and responsibility on parents to establish the educational groundwork for their children. Parents must possess the capacity to implement the appropriate parenting style, as parenting errors can have profound and challenging consequences to rectify. For instance, authoritarian parenting can cause children to become overly reliant on their parents and fearful of interacting with others. Parents who are incapable of instructing their children to contribute to household chores or prepare for daily necessities hinder the children's ability to engage in vital activities that foster independence (Taryani et al. 2019). Children must acquire knowledge and life experiences in order to attain independence. This includes the imperative to establish connections with others, build relationships, acquire cultural knowledge, and develop the capacity to protect themselves from potential threats. This also illustrates that the duration of auditory-verbal therapy alone is insufficient to facilitate children's attainment of social independence. Children require opportunities for socializing beyond their homes, as they may feel isolated and different from their peers, leading to increased withdrawal from social engagements (Sarry & Ervika 2018).

The social and interactive skills of children, as well as their ability to navigate within society, are of great significance. Parents must cultivate self-confidence in their children by encouraging them to independently accomplish tasks during each auditory-verbal therapy session, which can foster a sense of capability in both parents and children. It is essential to cultivate a sense of consistent discipline and responsibility in completing a task to promote robust language development (Komala 2015, Heriyanti 2020). Additionally, a study conducted by Silalahi (2014) indicated that parenting style is characterized by a consistent and continuous pattern of behavior used in interactions with children. This pattern of behavior may be perceived in both positive and negative ways by the children. The different perspectives on parenting styles can serve as a stimulus that molds children's mindset and independence levels. According to Alini & Indrawati (2020), optimal development of children's

additional traits is contingent upon the presence of a strong parent-child bond and the implementation of appropriate parenting practices. By fostering healthy and supportive relationships, particularly through the practice of democratic parenting in daily life, it is anticipated that children's independence can be nurtured and their personalities shaped accordingly.

Strength and limitations

This research encountered several limitations, notably in terms of time and a relatively small sample size. The limited duration of interviews prevented the collection of in-depth information required to explore the specific aspects of parenting styles that might impact the children's development of social independence, as well as factors that could influence the effectiveness of auditory-verbal therapy. Furthermore, the small sample size restricted our ability to draw meaningful comparisons among children at various age groups, potentially affecting the research's overall findings. Nevertheless, this research offers valuable insights for medical professionals, parents of the respondents, educators, and therapists. It sheds light on the significance of early detection and appropriate interventions for children with sensorineural hearing loss who experience speech delays. This research emphasizes that the duration of auditory-verbal therapy does not inherently guarantee success in fostering language skills and social independence in children. Instead, it depends on the effective implementation of the therapy. While the findings revealed no correlation between the duration of auditory-verbal therapy and the development of social independence, this research can serve as a catalyst for future research. It is suggested that future researchdelve into factors that exert a more significant influence on the development of social independence in children with speech delay and sensorineural hearing loss, as well as factors that more effectively contribute to the success of auditory-verbal therapy.

CONCLUSION

Both the age for early intervention and the duration of auditory-verbal therapy exert a noteworthy difference in a child’s language development with speech delay and sensorineural hearing loss. However, the observed children did not experience a notable difference in the development of social independence concerning their starting age and therapy duration. Future research is recommended to use a larger sample size to facilitate more detailed comparisons of children's development across various age groups. Researchers and participants are encouraged to allocate a more extensive timeframe for a thorough investigation of factors affecting children's development of social independence. Future research may also include other aspects, such as parenting styles and factors influencing the efficacy of auditory-verbal therapy.

Acknowledgment

The authors would like to extend their gratitude to Yayasan Aurica, Surabaya, Indonesia, for providing us with the opportunity to conduct this research effectively. We also wish to express our appreciation to all those who supported us in the process of writing this research article.

Conflict of interest

None.

Ethical consideration

This research received approval from the Health Research Ethics Committee at the Faculty of Medicine, Airlangga University, Surabaya, Indonesia, under authorization number 164/EC/KEPK/FKUA/2022, dated August 23, 2022.

Funding disclosure

None.

Author contribution

ZIP was responsible for concepting and designing the research, analyzing and interpreting the data, drafting the article, providing final approval of the article, overseeing the provision of research materials or patients, and collecting and assembling the data. ACR was responsible for drafting the article, critically revising the article for important intellectual content, providing final approval of the article, and offering administrative, technical, and logistic support. AS was responsible for drafting the article, conducting critical revisions for important intellectual content, providing statistical expertise, and offering administrative, technical, and logistic support. NP was responsible for drafting the article, conducting critical revisions for important intellectual content, and providing administrative, technical, and logistic support. NDA was responsible for the final approval of the article and providing administrative, technical, or logistic support.

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Original Research Report

HISTOLOGICAL STUDY OF THE RESTORATIVE EFFECT OF ROSELLE (*Hibiscus sabdariffa* Linn.) TEA ON THE DIGESTIVE ORGANS OF MONOSODIUM GLUTAMATE-INDUCED MICE (*Mus musculus* Linn.)

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ABSTRACT

Monosodium glutamate (MSG) is a commonly used synthetic additive for enhancing food flavor. Excessive use of MSG can cause cytotoxic effects, which disrupt the balance of oxidative stress and free radicals in the body, particularly in the human digestive system. Roselle (*Hibiscus sabdariffa*) is a plant with red petals renowned for its abundance of beneficial compounds, including polyphenols, flavonoids, anthocyanins, and other antioxidants that function as free radical antidotes. This study aimed to investigate the effect of administering roselle tea and determine the optimal dose for restoring the digestive organs of MSG-induced mice (*Mus musculus*). The research employed a completely randomized design with a random sampling method. A total of 25 mice were divided into five groups: a negative control group (K-) that received 0.3 mL of distilled water, a positive control group (K+) given 4 mg/g bw of MSG, and three treatment groups (P1, P2, and P3) administered with 4 mg/g bw of MSG along with varying doses of roselle tea (2.6 mg/g bw, 3.9 mg/g bw, and 5.2 mg/g bw, respectively). The treatment was orally administered via gavage for 30 days. The stomach, duodenum, and liver underwent histopathological examination using the paraffin method and hematoxylin-eosin staining. The observed parameters in the stomach and duodenum included necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation. Meanwhile, the parameters examined in the hepatic organs were necrosis, inflammatory cell infiltration, and cell degeneration. The Kolmogorov-Smirnov normality test and one-way analysis of variance (ANOVA) were employed to assess the normal distribution and homogeneity of the data. If the data exhibited a normal distribution, Duncan's post-hoc test was conducted. The results revealed significant differences among the groups ($p<0.05$). In conclusion, the administration of roselle tea effectively recovered the histological damage in the stomach, duodenum, and liver of MSG-induced mice.

Keywords: Digestive organs; healthy lifestyle; *Hibiscus sabdariffa* Linn.; monosodium glutamate (MSG)

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Article history
●Submitted 1/10/2023 ● Revised 11/11/2023 ● Accepted 28/11/2023 ● Published 10/12/2023

How to cite: Sistra KA, Wiratmini NI, Sukmaningsih AASA (2023). Histological Study of the Restorative Effect of Roselle (Hibiscus sabdariffa Linn.) Tea on the Digestive Organs of Monosodium Glutamate-Induced Mice (Mus musculus Linn.). Folia Medica Indonesiana 59 (4), 381-391, https://doi.org/10.20473/fmi.v59i4.51153

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

Highlights:
1. This original study examined the antioxidant compounds derived from naturally sourced Hibiscus sabdariffa calyx.
2. The findings demonstrated that roselle tea offers a viable and cost-effective solution for repairing histological damage to the digestive organs induced by monosodium glutamate.

INTRODUCTION

Presently, Indonesians have a preference for fast food as their everyday meal because it is easy to obtain, quickly served, convenient, and delicious. The delectable taste of fast food comes from the use of flavor enhancers (Pamelia 2018). Monosodium glutamate (MSG) is a synthetic flavor enhancer derived from glutamic acid. It is formed of L-glutamic acid that ionizes with sodium, resulting in the formation of L-glutamate sodium salt. This salt has the ability to enhance the flavor of food. The chemical structure of MSG is identical to that of glutamic acid. The only exception is that sodium substitutes one of the carboxyl groups, which typically contains hydrogen. Sodium-ionized carboxyl groups can stimulate the taste buds. This is what makes MSG, as a flavor enhancer, able to give

a savory taste (umami) to food (Kurtanty et al. 2018).

Glutamate contained in food is rapidly metabolized to become a source of energy. The absorption of glutamate from MSG occurs in the small intestine through an active transport system specific for the amino acid. During absorption in the small intestine, glutamate levels in the blood plasma increase. Glutamate consumption in large amounts leads to an increase in the level of glutamate in the body. This increase can cause heightened glutamate metabolism in the liver, which triggers the release of glucose, lactate, glutamine, and other amino acids (National Center for Biotechnology Information 2024). MSG is primarily absorbed in the small intestine by epithelial cells that line the intestinal mucosa. It is then dispersed throughout the body and undergoes metabolic processes in the liver. Finally, it is excreted by the kidneys through feces or urine (Zulfi et al. 2013, Airaodion 2019). The metabolism of MSG in the liver results in the production of a metabolite. Therefore, when the intake of glutamate exceeds the capacity of the liver to metabolize it, there will be a significant rise in glutamate levels (Onaolapo et al. 2016).

The estimated daily MSG consumption in Indonesia is approximately 0.6 g. However, the exact amount remains uncertain as consumers often indulge in foods with unknown MSG content, potentially leading to an increase in daily MSG consumption. While MSG consumption in moderate amounts is still considered safe, it is important to be aware of the permissible intake to avoid overconsumption. The Food and Drug Administration (FDA) of the United States and the World Health Organization (WHO) have established the maximum permissible limit of MSG at 120 mg/kg bw per day. Furthermore, several countries have set the maximum permissible consumption of MSG at 0.3–1 g per day (Yonata & Iswara 2016, Sulastri 2017). Prolonged and excessive consumption of MSG can lead to various side effects, such as increased glutamate levels in the blood, which may negatively affect the metabolism of the body. A high glutamate level also poses toxic effects on the central nervous system, leading to the interference of autonomic function and body metabolism, obesity, the disruption of reproductive system hormones, hepatotoxicity, and nephrotoxicity resulting from oxidative stress (Airaodion 2019).

Roselle (*Hibiscus sabdariffa* L.) has been used for its antioxidant, hypocholesterolemic, antiobesity, insulin-resistant activity reduction, antihypertensive, diuretic, and antimicrobial properties. It contains active compounds such as gossypetin, anthocyanin, and hibiscus glucoside, which have protective effects against degenerative diseases. Roselle ethanol extract comprises alkaloid, flavonoid, anthocyanin, phenolic, steroid, terpenoid, saponin, and tannin compounds (Gheller et al. 2017, Aryati & Rohadi 2020). Anthocyanins are flavonoid compounds that provide benefits to human health by protecting cells from damage caused by free radicals that enter the body. A previous study showed that administering 0.45 mL of dried roselle petal infusion twice per day effectively reduced cholesterol levels in hypercholesterolemic mice (Wahyuni 2015). A separate study investigated the potency of roselle leaf extract on the damaged liver of mice. The study found that the extract showed hepatoprotection effects capable of repairing the damage induced by natrium nitrite (NaNO2) (Sabri 2020). The detrimental effects of consuming excessive amounts of MSG become particularly evident in the digestive organs, which play a crucial role in processing food before it is converted into energy through metabolism. Hence, this study aimed to examine the effect of administering roselle tea and ascertain the most effective dose for repairing histological damage to the digestive organs of MSG-induced mice.

MATERIALS AND METHODS

This study was an experimental investigation using a completely randomized design and random sampling. The experimental animals used in the experiment were male mice aged 2 months with healthy conditions and a body weight of 20–30 grams. The total number of mice was 25, distributed evenly among five groups, with each group containing five mice (Wahid et al. 2017). The five groups consisted of a negative control group (K-), a positive control group (K+), and three treatment groups (P1, P2, and P3). The research was conducted at the Animal Physiology Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Udayana, Badung, Indonesia.

The negative control group (K-) was only given 0.3 mL of distilled water. The positive control group (K+) received 4 mg/g bw of MSG dissolved in 0.3 mL of distilled water, according to the instructions provided in a study by Maulida et al. (2013). The three treatment groups (P1, P2, and P3) were administered dried petals that had been prepared into roselle tea at varying doses. The dried petals used for infusion in the roselle tea were 2.6 mg/g bw, 3.9 mg/g bw, and 5.2 mg/g bw for groups P1, P2, and P3, respectively. The doses administered to the mice were determined by converting the amounts intended for human doses. Each dose of dried roselle petals was brewed in the same volume of water (0.5 mL) for the same duration of time (5 minutes). The experiment used MSG from brand "X" that was

purchased at a local market. Dried roselle petals from brand "XX" were acquired from a supermarket in Denpasar, Indonesia (8°41'04.1"S 115°12'56.7"E). These dried petals were processed to produce roselle tea for the treatment. The roselle plants were locally grown in Kediri, Indonesia (7°49'55.2"S 111°51'24.3"E).

Before administering the treatment, the mice underwent a seven-day acclimatization. They were housed in a plastic tub cage measuring 40x30x18 cm, with a top cover made of woven wire and a base paddded with rice husks. During acclimatization, all groups were provided standard feed (i.e., chicken pellets and ad libitum water). The room was maintained at a temperature of 25ºC with adequate air ventilation (Nugroho 2018). Following a seven-day period of acclimatization, the treatment started and continued for 30 days. The treatment was administered using the gavage technique, wherein the substance was orally fed through a feeding tube.

The administration of the treatment occurred following a fasting period of 14–18 hours. During the first week of the experiment, following a seven-day period of acclimatization, the mice in Groups P1, P2, and P3 were only given MSG. Starting from day 8, the mice in the treatment groups were administered roselle tea one hour after receiving a dose of MSG. On day 31, all the mice were anesthetized using ketamine and sacrificed by dislocating their necks. Afterwards, the stomach, duodenum, and liver organs were collected. The organs were washed in a 0.9% sodium chloride (NaCl) solution and fixed using a 10% neutral buffered formalin (NBF) solution (Maulida et al. 2013). The preparatory work was conducted at Denpasar Veterinary Center, Indonesia. This study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Universitas Udayana, Denpasar, Indonesia (No. B/60/UN14.2.9/PT.01.04/2023 on 28/03/2023).

By referring to the study by Yogini et al. (2021), the histological preparations of stomach, duodenum, and liver organs were observed at 100X and 400X magnifications using OptiLab Iris-2 Binocular Biological Microscope (Miconos, Indonesia) and OptiLab Advance V2 Viewer Software for Windows, version 2.21 (Miconos, Sleman, DIY, Indonesia). The paraffin method and hematoxylin-eosin (HE) staining were used in the histopathological examination. The number of damaged cells was counted from five fields of view using the Image Raster, a built-in application from OptiLab Advance V2 Viewer Software for Windows, version 2.21 (Miconos, Sleman, DIY, Indonesia). Parameters observed in the stomach and duodenum were necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation. In the hepatic organs, the parameters observed were necrosis, inflammatory cell infiltration, and cell degeneration. The data obtained were statistically analyzed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA). The normal distribution and homogeneity of the data were examined using the Kolmogorov-Smirnov normality test and one-way analysis of variance (ANOVA). It was followed by Duncan's post-hoc test if there were a normal distribution and significant differences ($p<0.05$). If the data distribution was not normal, the Kruskal-Wallis test was used. If the results were found to be significantly different ($p<0.05$), the analysis proceeded with the Mann-Whitney test (Masnunah et al. 2020).

RESULTS

Histopathological examination of the stomach organs

After administering MSG at a dose of 4 mg/g bw per day, histological examination of the stomach sections showed the presence of both normal and damaged cells, characterized by necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation. The normality of the data on the average number of damaged cells in the stomach was assessed using the Kolmogorov-Smirnov test. The stomach histology normality test yielded a normal distribution of the data ($p>0.05$). The normally distributed data were then subjected to a homogeneity test and a one-way ANOVA. The test results indicated statistically significant differences ($p<0.05$) among the treatment groups and control groups. Afterwards, the Duncan test was performed (Table 1).

The Duncan's post-hoc test results showed a significant difference ($p<0.05$) between the control and treatment groups. K- exhibited significant differences in comparison with K+. It was shown that K+ had the highest average count of damaged cells. This indicated that oral feeding of MSG at a dose of 4 mg/g bw per day resulted in stomach mucosal damage in the mice, as observed through histological examination. The damage was characterized by necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation.

The analysis of the stomach histological damage data showed statistically significant differences ($p<0.005$) between the positive control group (K+) and all the treatment groups (P1, P2, and P3). Statistically significant differences ($p<0.05$) were found in the levels of necrosis and inflammatory cell infiltration when comparing P1 to both P2 and P3.

However, there was no statistically significant difference between P2 and P3 (p>0.05).

Table 1. Results of the histopathological examination of the stomach after the administration of MSG and roselle tea.

Variables	Groups				
	K-	K+	P1	P2	P3
Necrosis (cells)	1.96±1.29[a]	24.93±1.62[d]	11.68±2.76[c]	6.91±1.33[b]	6.82±2.85[b]
Inflammatory cell infiltration (%)	1.23±0.19[a]	24.62±1.28[d]	12.41±2.43[c]	6.45±0.92[b]	5.76±1.04[b]
Villous erosion (%)	7.20±5.74[a]	33.60±7.07[c]	18.24±3.32[b]	13.28±2.81[ab]	12±3.62[ab]
Ephitelial desquamation (%)	7.68±5.02[ab]	18.56±3.98[c]	14.56±3.98[bc]	9.76±6.51[ab]	6.24±6.80[a]

Notes: The different superscripts behind the numbers in the same row indicate significant differences among the groups (p<0.05). K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).

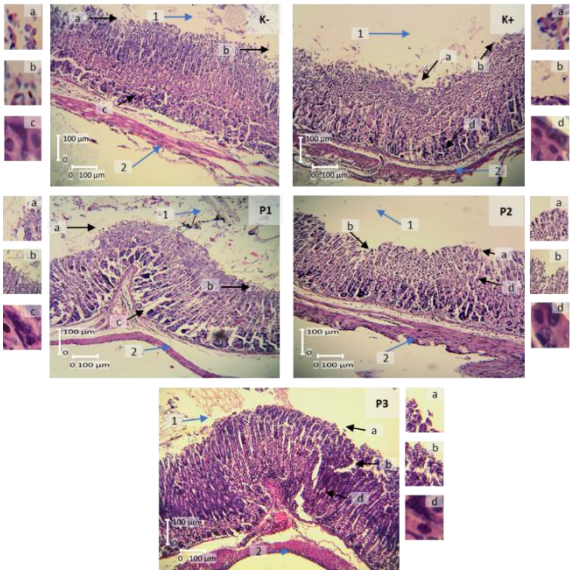

Figure 1. Stomach histological observation by HE staining (100X magnification).
Notes: a) Necrosis, b) inflammatory cell infiltration, c) villous erosion, d) epithelium desquamation; 1) lumen, 2) membrane basal. K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).

There was no significant difference in the decrease of villous erosion among P1, P2, and P3 (p>0.05). The decrease in epithelial desquamation damage was significantly different between P1 and P3 (p<0.05), however, there was no significant difference between P2 and either P1 or P3 (p>0.05). These results demonstrated that the administration of roselle tea in varying doses effectively mitigated stomach histological damage in mice. The histological damage to the stomach in each group is shown in Figure 1.

Histopathological examination of the duodenal organs

The histological examination of the duodenal sections showed a simultaneous presence of normal and damaged cells, characterized by necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation. This finding resembled the examination of the stomach organs following the ingestion of MSG at a dose of 4 mg/g bw per day. The data obtained from calculating the average number of damaged cells in the duodenum were examined for the normal distribution using the Kolmogorov-Smirnov test. The normality test revealed that the data on the histological duodenal sections had a normal distribution (p>0.05). The normally distributed data were evaluated using a homogeneity test and then proceeded with a one-way ANOVA. The test results yielded a statistically significant value of p<0.05 when comparing the treatment and control groups. Subsequently, the analysis continued with the Duncan test.

Table 2. Histopathological findings from the duodenum following the administration of MSG and roselle tea.

Variables	Groups				
	K-	K+	P1	P2	P3
Necrosis (cells)	0.99±0.41[a]	6.42±1.02[c]	3.80±0.90[b]	3.35±1.02[b]	1.37±0.76[a]
Inflammatory cell infiltration (%)	0.63±0.39[a]	4.21±1.56[d]	2.47±0.60[c]	1.95±0.76[bc]	1.23±0.32[ab]
Villous erosion (%)	6.08±6.76[a]	36.00±7.24[c]	18.72±4.85[b]	14.72±3.74[b]	5.12±5.20[a]
Epithelial desquamation (%)	9.92±8.05[ab]	29.28±5.95[c]	13.92±2.48[b]	7.36±6.36[ab]	4.96±4.75[a]

Notes: The different superscripts behind the numbers in the same row indicate significant differences among the groups (p<0.05). K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).

As shown in Table 2, the results of the Duncan's post-hoc test showed a significant difference (p<0.05) between the control and treatment groups. There was a significant difference between K- and K+. Regarding the level of organ damage, K+ exhibited the most severity compared to all the other groups. This indicated that orally administering MSG at a dose of 4 mg/g bw per day led to histological damage to the duodenal mucosa. Necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation were among the observed damages.

According to the analysis of the duodenal histological damage data, there were significant differences (p<0.05) between K+ and all the

treatment groups (P1, P2, and P3), as well as between P1 and P3. However, there was no significant difference between P1 and P2 ($p>0.05$). The comparison between P2 and P3 revealed statistically significant differences ($p<0.05$) in relation to necrosis and villous erosion, while no significant differences were found in terms of epithelial desquamation and inflammatory cell infiltration ($p>0.05$). Overall, these results demonstrated the effectivity of roselle tea in graded doses in reducing histological damage in the duodenum of mice. Figure 2 displays the histological damage observed in the duodenum, including necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation.

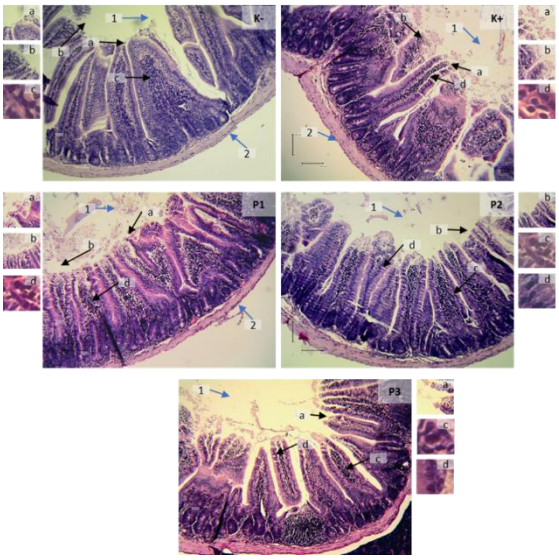

Figure 2. Histological observation of the duodenum using HE staining (100X magnification).
Notes: a) Necrosis, b) inflammatory cell infiltration, c) villous erosion, d) epithelium desquamation; 1) lumen, 2) membrane basal. K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).

Histopathological examination of the liver organs

The histological observation results revealed that both normal and damaged cells were present in the liver sections. The mice exhibited necrosis, inflammatory cell infiltration, and cell degeneration after receiving MSG at a dose of 4 mg/g bw per day. The data on the average count of damaged hepatic cells were analyzed using the Kolmogorov-Smirnov test. The results showed that the data had a normal distribution ($p>0.05$). The normally distributed data were then subjected to a homogeneity test and a one-way ANOVA. The result obtained was $p<0.05$, indicating a need for further analysis using the Duncan test (Table 3).

Table 3. Results of the liver histopathological examination following the administration of MSG and roselle tea.

Variables	Groups				
	K-	K+	P1	P2	P3
Necrosis (cells)	1.64±0.89^{a}	15.92±3.78^{d}	10.10±1.27^{c}	6.86±2.24^{b}	5.47±1.58^{b}
Inflammatory cell infiltration (%)	2.61±1.97^{a}	11.81±1.86^{d}	7.98±3.12^{c}	6.15±1.57^{bc}	4.73±1.90^{ab}
Villous erosion (%)	2.93±1.49^{a}	9.80±1.87^{d}	7.76±2.23^{cd}	6.78±2.23^{bc}	4.75±2.03^{ab}
Epithelial desquamation (%)	1.21±0.81^{a}	7.54±2.47^{d}	5.43±1.54^{cd}	3.82±2.71^{bc}	2.18±0.85^{ab}

Notes: The different superscripts behind the numbers in the same row indicate significant differences among the groups ($p<0.05$). K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).

The Duncan's post-hoc test results showed a significant difference ($p<0.05$) between the control and treatment groups. In addition, a significant difference was found between K- and K+. Similar to the results of the stomach and duodenal observations, K+ was found to suffer the highest damage across all groups. The oral administration of MSG at a dose of 4 mg/g bw per day caused histological damage to the hepatic tissue, as evidenced by the presence of necrosis, inflammatory cell infiltration, parenchymal degeneration, hydropic degeneration, and fatty degeneration.

The examination of the liver sections revealed significant differences ($p<0.05$) in the necrosis cell count between K+ and all the treatment groups (P1, P2, and P3), as well as between P1 and the other treatment groups (P2 and P3). Significant differences in inflammatory cell infiltration were observed between K+ and both P1 and P2, as well as between P1 and P3 ($p<0.05$). However, there was no significant difference between P2 and P3 ($p>0.05$). There were no significant differences observed in terms of parenchymal degeneration and hydropic degeneration between K+ and P1 ($p>0.05$). Additionally, there were no significant differences in hydropic degeneration and fatty degeneration between P1 and P2, as well as between P2 and P3 ($p>0.05$). Although there were few noticeable differences, the administration of roselle tea demonstrated its capacity to restore damage to the hepatocytes of the mice. The liver exhibited histological impairments such as necrosis, inflammatory cell infiltration, parenchymal degeneration, hydropic degeneration, and fatty degeneration, which can be seen in Figure 3.

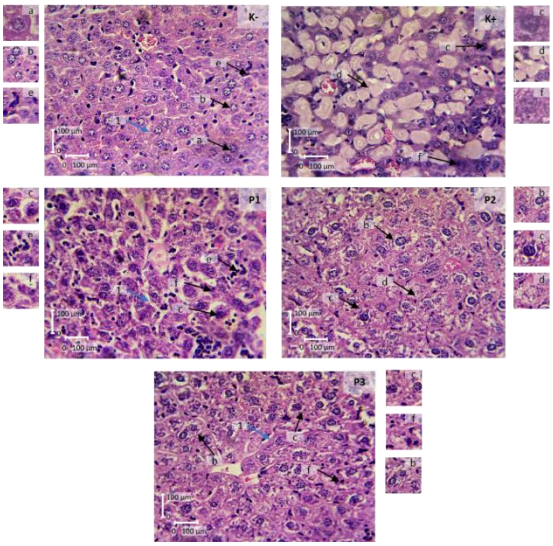

Figure 3. Liver histological observation by HE staining (400X magnification)
Description: a) Normal cells, b) hydropic degeneration, c) parenchymal degeneration, d) fatty degeneration, e) necrosis, f) inflammatory cell infiltration; 1) sinusoid. K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).

DISCUSSION

Histopathology of the stomach organs

The findings of this study demonstrated that the negative control group (K-), which solely received distilled water, exhibited the lowest average level of stomach mucosal damage. Although this group exhibited necrosis at a low level, it was deemed normal, as necrosis is a form of pathological cell death. The presence of inflammatory cell infiltration in this group indicated a cellular regeneration process. In addition, the presence of epithelial cell desquamation and villous erosion might be a stomach mucosal response due to a decrease in cytoprotective mucus secretion. The decrease in cytoprotection could be attributed to stressors arising from environmental conditions, such as adaptation sites, as well as the responses received by the experimental animals due to mechanical trauma to the stomach during daily administration of treatment via a feeding tube (Jahra et al. 2019).

The observation additionally demonstrated that the positive control group (K+) exhibited the highest average level of damage in the stomach mucosa following the administration of MSG at a dose of 4 mg/g bw per day. This damage was caused by high levels of glutamic acid in the body, which is derived from MSG. Increased levels of glutamic acid can produce free radicals in the body. Furthermore, high levels of glutamate affect the trycarbocylic acid (TCA) system, leading to an augmentation in alpha-ketoglutarate dehydrogenase activity. It can eventually stimulate the production of reactive oxygen species (ROS), hence amplifying oxidative stress (Jubaidi et al. 2019). Elevated concentrations of free radicals within the body will impair the weak polyunsaturated acids present in the cell membrane. This condition will cause the cell membrane to be fragile as well as allow the free radicals to enter the cytoplasm and damage the cell nucleus. Free radicals can change physiological functions in the body and trigger the inflammation reaction (Azab et al. 2019). Meanwhile, the influx of extracellular fluids into the cell can result in degeneration. If the cell is exposed to toxic substances for a long time, cell death or necrosis will occur. This is consistent with a previous study that demonstrated the potential harm of MSG consumption to the stomach organ (Yogini et al. 2021).

The findings from the treatment groups (P1, P2, and P3) suggest that roselle tea exerted a significant effect on the restoration of many forms of damage, including necrosis and inflammatory cell infiltration. However, roselle tea failed to show its effectiveness in repairing villous erosion and epithelial desquamation. Epithelial desquamation and villous erosion that occurred in the treatment groups resulted from the continuous administration of MSG. These impairments occurred simultaneously with the increase in free radicals, which can accelerate the inflammatory process and cause cell damage induced by oxidative stress. The heightened oxidative stress affects hydrochloric acid (HCL) secretion, decreases the production of prostaglandin hormones that function in the stomach mucosal barrier, induces ulcers in the mucosa, and disrupts gastric motility due to an increase in inflammatory mediators such as histamine and leukotriene (Riong 2022).

The administration of roselle tea at a dose of 2.5 mg/g bw resulted in minimal improvement in stomach mucosal damage in treatment group 1 (P1). A roselle tea dose of 3.9 mg/g bw produced a notable improvement in stomach mucosal damage in P2 compared to P1. However, with a roselle tea dose of 5.2 mg/g bw, P3 had less significant differences in terms of improvement in stomach mucosal damage compared to P2. This suggests that the moderate dose was quite effective in repairing stomach mucosal damage. Yet, when the higher dose was administered, it resulted in even greater restoration of the damage to the gastric mucosa, as seen by a decrease in the average level of damage. Furthermore, it also resulted in the lowest average level of epithelial desquamation. This might be due to the presence of antioxidant compounds in roselle tea, which can restore stomach cells (Amer et al. 2022). In this study, P3 exhibited the most minimal average level of stomach mucosal cell damage caused by MSG administration, with results

comparable to those of the negative control group. Therefore, the dose of 5.2 mg/g bw was considered the optimal dose of roselle tea with the capability to mitigate stomach mucosal damage.

Roselle tea offers a beneficial effect on reducing necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation due to its flavonoid content, which can counteract free radicals. Flavonoid is an antioxidant compound that protects the body by releasing one of its electrons to inhibit free radical activity (Nursheha & Febrianti 2015). The flavonoids contained in roselle petals are able to enhance the production of stomach mucus fluid. This fluid serves as a protective barrier for the stomach mucosa, shielding it from the harmful effects of acid, pepsin, and other substances such as MSG. This function is achieved by reducing histamine secretion from mast cells through the inhibition of the enzyme histidic decarboxylase. Roselle contains other antioxidants, such as saponins, which act as gastroprotective agents by activating the protection system of the stomach mucous membrane. A study conducted previously demonstrated that the thistle leaf extract also contains flavonoid, saponin, and tannin compounds, which have the ability to alleviate stomach histological damage in aspirin-induced rats (Riong 2022).

Histopathology of the duodenal organs

The histopathological examination of the duodenal organs showed that the negative control group (K-) had the lowest average level of mucosal damage. Necrosis damage in this group was within normal levels, as low levels of necrosis might indicate pathological cell damage. Inflammatory cell infiltration in this group might also indicate the process of duodenal cell regeneration. The presence of epithelial cell desquamation and villous erosion was seen as duodenal mucosal responses due to decreased mucus secretion as a cytoprotective mechanism (Jahra et al. 2019). On the other hand, the positive control group (K+) had the highest average level of damage following the administration of MSG.

The results of this study align with those of a study by Vincent et al. (2015), wherein MSG was found to induce damage to the duodenal mucosa. The mechanism of duodenal mucosal damage begins with epithelial desquamation, which refers to the detachment of epithelial cells from the tissue surface. In the duodenum, this process serves as a defense mechanism in response to irritants, aiming to prevent additional harm (Alfina et al. 2022). The elevated concentrations of glutamic acid in the body, which are generated by MSG, are responsible for this damage. The mechanism of damage caused by MSG can be attributed to the presence of free glutamate, which stimulates stomach acid secretion. Meanwhile, villous erosion is an advanced form of damage when the duodenum experiences partial loss of epithelium in its mucosal layer (Sulastri et al., 2018). It occurs because Brunner's glands do not produce alkaline mucus that can protect the duodenal wall against gastric acid secretion (Putra et al. 2021).

The results from all the treatment groups (P1, P2, and P3) demonstrated that roselle tea effectively decreased necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation. The continued administration of MSG in these groups led to damage characterized by epithelium desquamation and villous erosion, which in turn caused an increase in free radicals. Oxidative stress occurs when highly reactive free radical molecules continuously generate free radicals, leading to cellular damage. Stress in the digestive tract can reduce mucosal blood flow, which can disrupt the integrity of the mucosal barrier by inhibiting Brunner's glands (Putra et al. 2021). The duodenal mucosa can be damaged by prolonged and excessive release of gastric acid, leading to an inflammatory process that can harm the structure of the duodenal mucosa.

The administration of roselle tea at doses of 2.5 mg/g bw and 3.9 mg/g bw had a positive effect on improving duodenal mucosal conditions. However, the administration of roselle tea at a dose of 5.2 mg/g bw provided the most significant improvement in the damage caused by MSG, with results comparable to those of the negative control group. The administration of roselle tea at a higher dose resulted in the most minimal damage to the duodenal mucosal cells. Therefore, a roselle tea dose of 5.2 mg/g bw was determined to be the optimal dose that has the potential to reduce duodenal mucosal damage. On a side note, it is noteworthy that the duodenal mucosal damage was less severe in comparison to the damage observed in the stomach. MSG is primarily processed in the stomach with the help of gastric digestive sap, making the stomach exposed to a significant quantity of toxic substances. The toxic substances are then transferred and absorbed by the duodenum in smaller amounts (Vincent et al. 2014).

Roselle tea is rich in natural antioxidants, including phenolic compounds such as anthocyanins, gossypetin, vitamin C, vitamin B, and vitamin D. Additionally, it has polyphenol and flavonoid compounds, which act as antioxidants by binding to free radicals, therefore protecting cells and preventing lipid peroxidation. Hardiningtyas et al. (2014) suggest that flavonoid antioxidants function by absorbing and inhibiting the regeneration of ROS

while also indirectly enhancing the activity of cellular antioxidant enzymes. Roselle possesses anthocyanins and polyphenols that enhance the mucosal barrier of the digestive tract by proliferating beneficial digestive microflora bacteria, such as Lactobacillus spp. and Bacillus spp. (Amer et al. 2022).

Histopathology of the liver organs

The histopathological observation of the hepatic organs revealed that the negative control group (K-) exhibited a mixture of normal and damaged cells. Normal hepatocytes typically have polyhedral, round, or oval shapes with hepatocyte plates. Other characteristics of normal hepatocytes are bright red cytoplasm, white sinusoids, and an intact appearance (Anggraeny 2014). In this study, the negative control group displayed necrosis, inflammatory cell infiltration, and degeneration, but to a lesser extent than the positive control group and the treatment groups. The observed organ damage might be attributed to the pathological process, when all cells in the body undergo cell death as a result of toxic substances or certain factors, followed by a regenerative phase that produces new cells.

The positive control group (K+) had the highest average level of hepatocyte damage. The statistical analysis revealed a significant prevalence of cell degeneration and necrosis. The hepatocyte damage resulted from the repeated administration of MSG, which heightened the amount of radical chemicals generated by the secondary metabolism of glutamic acid, leading to the production of hydrogen peroxide. Hydrogen peroxide can react with chemicals present in the body and form reactive hydroxyl radicals. The formation of hydroxyl radicals induces the production of lipid peroxides, which in turn disrupt the integrity of the cell membrane. Consequently, the cell structure becomes abnormal and impaired (Ayala et al. 2014).

Hepatic tissue damage due to continuous administration of MSG begins with the process of cell degeneration. Excessive MSG consumption can lead to the influx of a significant volume of extracellular fluid into the cytosol, causing hepatocytes to swell (Maulida et al., 2013). Prolonged MSG consumption can also accumulate the substance in the hepatic organ, which is responsible for filtering toxic substances that enter the body. This accumulation can harm hepatocytes due to the effects of free radicals caused by MSG (Anggraeny 2014). Once the cell membrane is damaged, the effects of the toxic substance can extend to the nucleus, resulting in structural abnormalities and the onset of necrosis. Cell death, also known as necrosis, is the result of persistent degeneration that harms the cell membrane system, causing the cell to lyse and perish (Wijaya et al., 2014). Free radicals can lead to cell membrane damage, which in turn triggers an inflammatory response characterized by inflammatory cell infiltration. The liver inflammation observed in this study was an immune response triggered by the toxic properties of MSG. However, hepatocytes could regenerate rapidly because the damage caused by the toxic substance was reversed with the administration of antioxidants that possess hepatoprotective agents, such as flavonoids, polyphenols, saponins, and alkaloids (Gebremedhin et al. 2020).

The administration of roselle tea at a dose of 2.5 mg/g bw was unable to repair hepatocyte damage. This was indicated by the damage levels of parenchymal degeneration and hydropic degeneration in treatment group 1 (P1) that were found to be similar to those of the positive control group (K+). Conversely, the administration of roselle tea at a dose of 3.9 mg/g bw resulted in an improvement in hepatocyte damage. However, there was no significant difference observed compared to the administration of the lowest dose in terms of parenchymal degeneration, hydropic degeneration, and inflammatory cell infiltration. Overall, the administration of roselle tea at a dose of 5.2 mg/g bw generated an improvement in hepatocyte damage, which was not significantly different compared to the moderate dose but significantly different than the lowest dose. This dose also resulted in the lowest average level of damage, such as fatty degeneration, because it contains antioxidants that can restore hepatocytes (Amer et al. 2022). Additionally, the results of this dose administration were similar to those of the negative control group. Hence, it was concluded that a roselle tea dose of 5.2 mg/g bw was the most effective in mitigating liver deterioration and hepatocyte damage.

Water-soluble extracts of roselle petals contain protocatechuic acid and anthocyanins, which can protect the liver from damage. A study conducted by (Adeyemi et al. 2014) has provided support for this concept. The study demonstrated that the administration of roselle extract effectively improved the liver fibrosis in diabetic rats induced by streptozotocin. The observed effectivity of roselle extract might be partly related to its antioxidant properties, including protocatechuic acid, anthocyanins, and flavonoids, which help prevent peroxidative liver damage. In a study conducted by (Zuraida et al. 2015), it was found that giving rats higher doses of roselle extract was more effective in enhancing the antioxidant activity of ascorbic acid compared to the use of lower doses. This increased antioxidant activity was beneficial for inhibiting lipid peroxidation caused by free radicals, leading to a decrease in malondialdehyde (MDA) levels and liver necrosis in rats exposed to

carbon tetrachloride (CCL4).

Strength and limitations

The administration of roselle tea (*Hibiscus sabdariffa* Linn.) at graded doses can effectively restore histological damage in the stomach, duodenum, and liver of mice (*Mus musculus*) induced by monosodium glutamate (MSG). A high dose provides the most optimal outcomes in terms of ameliorating digestive organ damage. Further comprehensive study is required to investigate the potential effect of administering roselle tea on the histological organ damage associated with the administration of substances other than MSG. The evaluation of roselle tea as an herbal remedy shall be considered.

CONCLUSION

The administration of roselle tea (*Hibiscus sabdariffa* Linn.) at graded doses effectively restored histological damage in the stomach, duodenum, and liver of mice (*Mus musculus*) induced by monosodium glutamate (MSG). A high dose provided the most optimal outcomes in terms of ameliorating digestive organ damage. A further comprehensive study is required to investigate the potential effect of administering roselle tea on the histological organ damage associated with the administration of substances other than MSG. The evaluation of roselle tea as an herbal remedy shall be considered.

Acknowledgment

The authors would like to thank the lecturers and staff of the Animal Physiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Udayana, Badung, Indonesia, as well as the staff of Denpasar Veterinary Center for their valuable guidance and assistance.

Conflict of interest

None.

Ethical consideration

This study received ethical clearance from the Animal Ethics Committee of the Faculty of Veterinary Medicine, Universitas Udayana, Denpasar, Indonesia, with registration No. B/60/UN14.2.9/PT.01.04/2023 on 28/03/2023.

Funding disclosure

None.

Author contribution

KAS conceptualized the study, analyzed and interpreted the data, drafted the article, provided the study materials, statistical expertise, and administrative support, and collected and assembled the data. NIW conceptualized the study, developed the methodology, critically revised the article for important intellectual content, provided statistical expertise, and provided technical and logistic support. AASAS provided validation for the study and provided statistical expertise. All authors have read and approved of the final version of the article for publication.

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Original Research Report

ASCORBATE AND ALPHA-TOCOPHEROL MITIGATE TOXIC PATHOLOGICAL CHANGES IN ADULT WISTAR RATS EXPOSED TO CYPERMETHRIN

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ABSTRACT

The excessive and uncontrolled use of pyrethroids, such as cypermethrin (CP), for pest control in Nigeria could adversely affect humans. This study aimed to investigate the oxidative stress response to cypermethrin exposure, focusing on measuring the parameters (i.e., malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT)) and the potential therapeutic effects of single and co-administration of ascorbate and alpha-tocopherol. The lungs and hearts of the animals were histologically examined for cypermethrin-induced cytopathic changes. Twenty-five adult male Wistar rats weighing 180–200 g were randomly assigned to five groups, each consisting of five animals. Group I was the control group that was not subjected to any treatment. Group II was orally exposed to cypermethrin at a dosage of 10 mg/kg bw without any additional treatment. Groups III, IV, and V received cypermethrin at standard doses of 10 mg/kg bw and were orally administered with ascorbate (5,000 mg/kg bw), alpha-tocopherol (3,000 mg/kg bw), and a co-administration of ascorbate (5,000 mg/kg bw) and alpha-tocopherol (3,000 mg/kg bw), respectively. The animals were euthanized after 28 days, and samples were processed for histological analysis using hematoxylin and eosin staining. Analysis of variance (ANOVA) and Duncan's multiple range test were used to compare categorical variables of the biochemical parameters and determine the levels of MDA, SOD, GPx, and CAT. The data analysis revealed that the cypermethrin-exposed, untreated rats had elevated MDA levels and a concurrently marked decrease in SOD, GPx, and CAT activities ($p<0.05$). Additionally, the histopathological examination of the organs indicated inflammation and congestion. The co-administration of ascorbate and alpha-tocopherol restored the biochemical parameters more effectively compared to when the substances were administered individually. In conclusion, co-administration of ascorbic acid and alpha-tocopherol ameliorates cypermethrin-induced oxidative damage more effectively than a single administration of either substance. This may be due to the synergistic antioxidant properties of the substances.

Keywords: Agricultural practices; cypermethrin; oxidative stress; healthy lifestyle; farmers

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Article history
●Submitted 11/9/2023 ● Revised 12/10/2023 ● Accepted 27/11/2023 ● Published 10/12/2023

How to cite: Adeniyi TD, Moronkeji A, Ralph-Okhiria OFR, et al (2023). Ascorbate and Alpha-Tocopherol Mitigate Toxic Pathological Changes in Adult Wistar Rats Exposed to Cypermethrin. Folia Medica Indonesiana 59 (4), 392-399, https://doi.org/10.20473/fmi.v59i4.49611

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

Highlights:
1. This study provides insight into the detrimental effects of cypermethrin exposure on the human cardiopulmonary system as well as the beneficial roles of ascorbate and alpha-tocopherol in cypermethrin-induced toxicity.
2. This study investigated the effectiveness of ascorbate and alpha-tocopherol as affordable vitamin supplements for Ondo farmers to protect against cypermethrin-induced oxidative damage.
3. The co-administration of ascorbate and alpha-tocopherol mitigates cypermethrin-induced oxidative damage better than a single administration of either subtance.

INTRODUCTION

Pyrethrins are insecticidal compounds obtained from the flowers of the plant *Tanacetum cinerariaefolium*, which is also known as *Chrysanthemum cinerariaefolium* or *Pyrethrum cinerariaefolium*. Pyrethroid insecticides, which can be produced from the plant, are highly effective against a wide range of pests found on both animals and livestock (Ensley 2018). When sprayed and exposed to the environment, pyrethroids (e.g., cypermethrin) can come into contact with the skin, nostrils through inhalation, and mouth through ingestion. These substances have been shown to exert negative effects on both animals and humans (Kaur & Singh 2021). Humans can be exposed to

pesticides in a variety of ways, particularly in environments that encompass farmlands and water bodies. The extent to which cypermethrin affects human health and the environment is determined by the amount of the substance present and the duration and frequency of exposure (Ye et al. 2017). In Nigeria, the uncontrolled use of pyrethroid pesticides has constituted a public health problem. Pyrethroids are rapidly metabolized, resulting in the production of easily excretable metabolites. This characteristic allows them to be a safer alternative to organochloride pesticides. However, this does not fully deny the fact that pyrethroids have the potential to bioaccumulate in various organs (Atere et al. 2021).

Cypermethrin is a type II pyrethroid commonly used in agriculture and households as an insecticide. Despite its advantageous uses, numerous pieces of evidence have indicated that exposure to cypermethrin at either acute or chronic levels may lead to health problems, including respiratory issues, cancer, and neurological diseases (Huang et al. 2018). Although pyrethroids are considered safer than relatively carcinogenic organochloride pesticides, they have been found to induce some adverse effects in mammals. Earlier studies have reported that acute and chronic exposure to pyrethroids may result in neurotoxicity, increased oxidative stress, and a wide variety of toxic effects such as hepatotoxicity and nephrotoxicity (Chrustek et al. 2018, Bouabdallah et al. 2021, Atere et al. 2021). A study by Sandhu et al. (2010) indicated the presence of toxic symptoms, which ranged from mild to moderate, and behavioral changes in rats orally administered with repeated doses of cypermethrin at 5 and 20 mg/kg/day for 30 days. The histopathological examination of the cardiac tissues did not reveal any cytopathic changes in rats receiving lower cypermethrin doses. Meanwhile, hemorrhages, disruption in the branching structure with loss of striations, and early necrotic changes in the myocardium were evident in rats receiving higher cypermethrin doses.

Cypermethrin may predispose local farmers and individual users to its effects via inhalation and the oral route. This sometimes occurs inadvertently when the farmers consume contaminated food and drink while working. Previous research has shown that the cardiopulmonary system is susceptible to toxicity from pesticide exposure. Additionally, it is a well-established fact that pesticides, such as cypermethrin, have the propensity to bioaccumulate, particularly in the lungs and heart (Ratanachina et al. 2020, Yu et al. 2022). Against this background, we aimed to examine the effect of ascorbate and alpha-tocopherol on cypermethrin-induced oxidative damage in adult male Wistar rats. The concern associated with uncontrolled pyrethroid exposure necessitated this study to determine whether ascorbate and alpha-tocopherol can mitigate toxic pathological changes caused by cypermethrin exposure.

MATERIALS AND METHODS

A total of 25 adult male Wistar rats weighing around 180–200 g was obtained from the Animal House, Faculty of Basic Medical Sciences, University of Medical Science, Ondo, Nigeria. The rats had a two-week acclimation period and were adequately housed in a well-ventilated and clean space. They were properly fed with standardized rat pellets sourced from the university's animal holding facility. Daily monitoring was carefully carried out throughout the experiments. The experimental animals were handled in accordance with the International Humane Animal Care Standards (Hau & Schapiro 2013).

The rats were divided into five groups consisting of five rats each. The rats in Group I served as controls, meaning they were unexposed to cypermethrin and did not receive any treatment. Group II consisted of rats that were orally exposed to cypermethrin at a standard dosage of 10 mg/kg bw without any treatment given. Groups III, IV, and V were administered cypermethrin at standard doses of 10 mg/kg bw. Group III was treated with ascorbate at a dose of 5,000 mg/kg bw. Group IV received alpha-tocopherol at a dose of 3,000 mg/kg bw. Meanwhile, Group V received a co-administration of ascorbate (5,000 mg/kg bw) and alpha-tocophero (3,000 mg/kg bw). Cypermethrin was administered twice a week, while ascorbate and alpha-tocopherol were given daily for 28 days (Hassan 2019, Oladele et al. 2022).

The experiments used a cypermethrin-based pesticide of commercial grade with an effect concentration (EC) of 10%. Cypermethrin [(RS)-cyano-(3-phenoxyphenyl) methyl-(IRS)-cis-trans-3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropane carboxylate] with the trade name Avestrin® was manufactured in Lagos, Nigeria, by Harvestfield Industries Ltd. The substances used to mitigate the effects of cypermethrin exposure were ascorbic acid and alpha-tocopherol (Pisoschi & Pop 2015). The alpha-tocopherol capsules manufactured in Dabhel, India, by Olive Healthcare were purchased from Ever Destiny Pharmaceuticals Ltd., Lagos, Nigeria. Each soft gelatin capsule contained 1000 IU of vitamin E acetate, as specified by the United States Pharmacopeia (USP) Reference Standard. Vitamin C tablets, produced in Lagos, Nigeria, by Chemo-Pharma Laboratories Ltd. and registered with the National Agency for Food and Drug Administration and Control (NAFDAC) under the number 04-3486,

were purchased from Uche Care Pharmaceuticals, Ondo, Nigeria. Each tablet contained 100 mg of ascorbic acid, in accordance with the British Pharmacopoeia (BP) Reference Standard.

Upon completion of the experiments, the rats were euthanized by means of cervical dislocation. The hearts and lungs of the rats were immediately excised to collect specimens, which were then transferred into freshly prepared 0.1 mol/L phosphate buffered saline (PBS) with a pH of 8.0. The specimens were homogenized and centrifuged at 3,000 revolutions per minute for 20 minutes at 4°C. The obtained supernatants were stored at -80°C for the analysis of biochemical markers, i.e., malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). The histopathological samples were transferred into freshly prepared 10% neutral buffered formalin and processed for microscopic analysis following the method outlined by (Suvarna et al. 2019).

As described by Kalinovic et al. (2021) and Oladipo et al. (2018), the lung and heart tissue homogenates were prepared for the analysis of malondialdehyde (MDA). The biochemical indicators were analyzed using the enzyme-linked immunosorbent assay (ELISA) following the protocols provided in the kits. The ELISA kits were manufactured in the USA by Elabscience Biotechnology, Inc. The ELISA analysis was performed to assess the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). The detection ranges for CAT, SOD, and GPx were 1.12-150 U/mL, 0.2-14.4 U/mL, and 17.7-518.32 U/mL, respectively (Kang et al. 2020, Huang et al. 2022, Li et al. 2023).

The prefixed lungs and heart tissues were prepared with an automatic tissue processor machine before being observed under a light microscope. The stained sections were examined using a binocular light microscope (model XSZ-107BN, No. 071771, Olympus, Japan), while micrographs were taken at 100X magnification with a digital camera (model Easyshare C183, Kodak, USA) (Suvarna et al. 2019, Akinpelu et al. 2023). The collected data were subjected to statistical analysis using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA). The categorical variables were compared using analysis of variance (ANOVA) and Duncan's multiple range test. A value of $p<0.05$ was considered statistically significant, with a confidence level of 95%.

RESULTS

The histopathological examination of the lung section of the control rats that were not exposed to any substances showed no abnormalities. The bronchioles appeared normal, and the intralveolar spaces were devoid of inflammation or congestion. This indicated the absence of pulmonary toxicity, as cypermethrin was not administered (Figure 1a). The untreated rats that were exposed to cypermethrin exhibited peribronchiolar inflammation and congestion, coupled with a focal area of inflammation where the intra-alveolar spaces collapsed. This indicated that exposure to cypermethrin caused pulmonary toxicity in these rats (Figure 1b). The bronchioles of the rats treated with ascorbate did not show signs of inflammation. However, there was mild inflammation in the intraalvolar spaces (Figure 1c). The rats treated with alpha-tocopherol had a normal bronchiole, with a mild infiltration of the intra-alveolar spaces by mononuclear inflammatory cells (Figure 1d). On the other hand, the rats co-administered with ascorbate and alpha-tocopherol exhibited a normal bronchiole with mildly inflamed intra-alveolar spaces (Figure 1e).

The cardiac section of the unexposed control rats exhibited a normal appearance, with a typical myocardial layer and healthy-looking myocytes. This result indicated that there was no cardio-toxicity, considering cypermethrin was not provided to these rats (Figure 2a). The cypermethrin-exposed rats that did not receive any treatment had a mildly congested myocardium, suggesting a presence of cardiotoxicity induced by cypermethrin exposure (Figure 2b). The cypermethrin-exposed rats that were treated with ascorbate showed a congested myocardial layer (Figure 2c), while those treated with alpha-tocopherol had congested myocardium (Figure 2d). The co-administrative treatment using ascorbate and alpha-tocopherol resulted in a normal myocardium devoid of pathological lesions (Figure 2e).

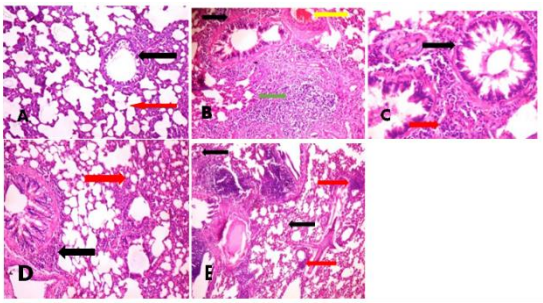

Figure 1. (a) Normal bronchiole (black arrow) and intra-alveolar spaces (red arrow) in Group I; (b) peribronchiolar inflammation and congestion (black arrow), inflammed and collapsed intra-alveolar spaces (green arrow), and mild vascular congestion (yellow arrow) in Group II; (c) normal bronchiole (black arrow) and mildly infiltrated intra-alveolar spaces (red arrow) in Group III; (d) normal bronchiole (black arrow) and infiltrated intra-alveolar spaces (red arrow) in Group IV; (e) normal bronchiole (red arrows), mildly infiltrated intra-alveolar spaces in Group V (100X magnification).

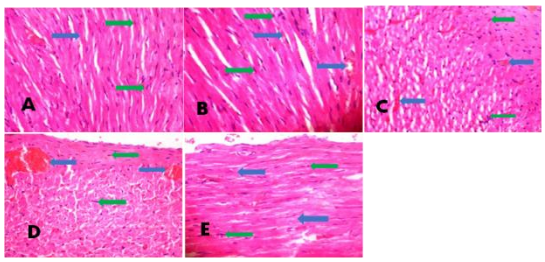

Figure 2. (a) Normal myocardial layer (blue arrow) and myocytes (green arrow) in Group I; (b) mildly congested myocardium (blue arrow) and myocytes (green arrow) in Group II; (c) congested myocardium and myocytes (green arrow) in Group III; (d) congested myocardium (blue arrow) and myocytes (green arrow) in Group IV; (e) normal myocardium (blue arrow) and myocytes (green arrow) in Group V (100X magnification).

The biochemical evaluation of the oxidant and antioxidant parameters demonstrated the effect of cypermethrin on the lung tissue homogenates and the variations in the levels of the oxidant MDA and the antioxidants SOD, GPx, and CAT among the different groups. The rats that were exposed to cypermethrin exhibited significantly higher levels of MDA compared to the unexposed control rats. Conversely, the rats that were treated with a single administration of either ascorbate or alpha-tocopherol showed significantly decreased levels of MDA. The co-administration of ascorbate and alpha-tocopherol resulted in a significant decrease in MDA levels compared to the other treatment groups (Table 1). The SOD levels were reduced significantly in the cypermethrin-exposed rats that did not receive any therapy. Conversely, the co-administration of both vitamins resulted in superior treatment outcomes compared to the single administration of either ascorbate or alpha-tocopherol. The levels of GPx and CAT significantly decreased in the rats exposed to cypermethrin, compared to the control rats that were not treated as well as the other groups receiving vitamin treatment. However, a marked increase in antioxidant levels was observed in the rats that received both ascorbate and alpha-tocopherol, indicating the therapeutic effect of co-administering these two substances (Table 1).

The biochemical evaluation of the effect of cypermethrin on heart tissue homogenates revealed a significant rise in the oxidant MDA levels in the treatment groups compared to the control group that was not exposed to the substance. Additionally, the levels of the antioxidants SOD, GPx, and CAT were found to be significantly different across the treatment groups. The rats that were given a combination of ascorbate and alpha-tocopherol showed the most significant reversal of the oxidative damage. The levels of SOD, GPx, and CAT in all treatment groups were significantly lower compared to the controls. However, the co-administration of ascorbate and alpha-tocopherol more effectively restored the levels of SOD, GPx, and CAT in comparison to the other groups receiving different treatments (Table 2).

DISCUSSION

Pesticides, including pyrethroids, are beneficial for pest control in various environments, such as farmlands or households. However, the unregulated use of pyrethroids potentially induces oxidative stress and cardiopulmonary toxicity when food and water sources for human consumption become contaminated (Shaffo et al. 2018, El-Nahhal & El-Nahhal 2021). The experiment in this study used oral administration of cypermethrin to simulate a major route of contamination, specifically via the ingestion of contaminated food and drink. This means of cypermethrin exposure frequently occurred among local farmers who made use of the

Table 1. Biochemical parameters in the lung tissue homogenates across the various groups.

Parameters	Group I	Group II	Group III	Group IV	Group V
MDA (µmol/mg protein)	0.3482±0.08446^{c}	3.2594±0.46949^{a}	1.5796±0.37563^{b}	1.4726±0.60105^{b}	0.6396±0.25314^{c}
SOD (U/mg protein)	7.874±0.92813^{a}	3.347±0.14764^{c}	4.2912±0.18541^{bc}	5.235±0.70549^{b}	6.846±1.62827^{a}
GPx (µmol/min/mg protein)	94.5962±2.81905^{a}	17.1548±1.92755^{d}	47.9164±5.44814^{c}	51.2416±6.25599^{c}	72.6032±5.91337^{b}
CAT (mmol/mg protein)	17.728±2.08951^{a}	4.4058±1.79837^{c}	7.8166±1.85901^{b}	9.2802±1.20953^{b}	15.4152±3.66585^{a}

Notes: The mean±SD values with different superscripts represent significant differences at a 5% level, with a>b>bc>c>d. The significant values for each superscript are as follows: a=0.000, b=0.015, bc=0.025, c=0.034, d=0.043.

Table 2. Biochemical parameters in the heart tissue homogenates across the various groups.

Parameters	Group I	Group II	Group III	Group IV	Group V
MDA (µmol/mg protein)	0.1336±0.03231^{c}	1.4544±0.30831^{a}	0.6886±0.1459^{b}	0.6266±0.21744^{b}	0.2458±0.09742^{c}
SOD (U/mg protein)	3.0312±0.35739^{a}	0.5063±0.22603^{c}	1.3362±0.31807^{b}	1.6884±0.58682^{b}	2.6356±0.62685^{a}
GPx (µmol/min/mg protein)	37.1448±2.01953^{a}	8.3302±1.69969^{d}	16.1076±1.66911^{c}	16.4278±2.32259^{c}	34.142±2.86812^{b}
CAT (mmol/mg protein)	6.8248±0.80443^{a}	1.4958±0.32051^{c}	3.408±0.4293^{b}	3.6814±0.25831^{b}	5.9344±1.41127^{a}

Notes: The mean±SD values with different superscripts represent significant differences at a 5% level, with a>b>c>d. The significant values for each superscript are as follows: a=0.001, b=0.019, c=0.028, d=0.040.

pesticide for farming. The dose for the administration of cypermethrin was determined by a thorough analysis of multiple prior studies (Abdus Sallam et al. 2020, Akinpelu et al. 2023).

The findings of tests conducted on rats by Akorede et al. (2020) and Abdel-Razik et al. (2021) demonstrated that the administration of cypermethrin at doses between 5 mg/kg bw and 20 mg/kg bw could induce oxidative damage. Therefore, we opted for a dosage of cypermethrin within this range to be applied in this experiment. The dose was also determined by considering the ratio of the median lethal dose (LD50). The LD50 of cypermethrin was determined to be 250 mg/kg bw (Bouabdallah et al. 2021). The determination of the cypermethrin doses to be administered was conducted as a preventive measure against unnecessary animal mortality.

We reasoned that the contamination we attempted to replicate was sufficient, given that the farmers did not directly consume the chemicals but were inadvertently exposed through food and drink contamination instead. In addition, the farmers used pesticides not on a daily basis but rather on a seasonal basis. As a result, we deduced that the chosen dose would be adequate to induce the expected toxic effects.

Studies have suggested that vitamins can act as a form of therapy to tackle oxidative stress. Ascorbate and alpha-tocopherol are among these beneficial vitamins (Pisoschi & Pop 2015, Wang & Dong 2018, Akinpelu et al. 2023). Oxidative stress arises from an imbalance between the presence of oxidants in the body and the body's ability to combat them. In the presence of oxidants, the body needs to be able to regulate reactive oxidants or repair the resulting damage produced by them (Choudhury & MacNee 2017). In this study, we investigated the oxidative stress response in adult male Wistar rats exposed to cypermethrin, which is a type of pyrethroid often used by farmers in Ondo, Nigeria. Additionally, we evaluated the protective effects of ascorbate and alpha-tocopherol when administered singly or in combination.

This study examined the histological changes in the lungs and hearts of the cypermethrin-exposed rats to identify the cytopathic alterations induced by cypermethrin. The findings obtained from the biochemical and histological analyses provide valuable insights into the extent of oxidative stress caused by cypermethrin exposure and the potential ameliorative effects of the antioxidant treatments. The control group that was not subjected to any interventions showed no signs of oxidative distress or response throughout the experiment. This finding was supported by the absence of a significant increase in MDA levels as well as the steadiness of normal SOD, GPx, and CAT levels without any noticeable decrease. These results are consistent with the findings reported by (El-Okda et al. 2017).

The histoarchitectural analysis of the control rats revealed that their lungs were devoid of pathological lesions, as typified by normal bronchioles and alveolar spaces. Similarly, the heart tissues exhibited normal epicardial and myocardial layers, and the myocytes were normal without any signs of inflammation or congestion. This was consistent with the findings reported by Ghazouani et al. (2020). The results obtained from the cypermethrin-unexposed control group established a baseline for comparing them with the treatment groups. This enabled a comprehensive evaluation of cypermethrin-induced oxidative stress and the protective effects of ascorbate and alpha-tocopherol.

The cypermethrin-exposed, untreated rats exhibited significant alterations in both biochemical and histological parameters, indicating damage induced by oxidative stress. Biochemically, elevated levels of MDA were observed in this group, suggesting increased lipid peroxidation and oxidative damage to the cellular membranes (Hassan 2019). Concurrently, the activities of essential antioxidant enzymes, i.e., SOD, GPx, and CAT, were significantly reduced. This indicated an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms. In a previous study conducted by Ghazouani et al. (2020), it was observed that cypermethrin exposure reduced the levels of SOD, GPx, and CAT. The histopathological analysis of lung tissues in the group exposed to cypermethrin revealed inflammation and congestion, which were indicative of cypermethrin-induced lung injury.

The heart tissue examination revealed mild congestion, signifying the onset of cardiotoxicity due to cypermethrin exposure. In contrast, the group solely treated with ascorbate demonstrated improved biochemical and histological parameters, suggesting a potential ameliorative effect against cypermethrin-induced oxidative stress. The biochemical analysis revealed a decrease in MDA levels, indicating the attenuation of lipid peroxidation. This finding is consistent with the study conducted by Akorede et al. (2020), who documented a significant reduction in MDA levels in adult male Wistar rats treated with ascorbate to alleviate carbamazepine-induced oxidative stress. Furthermore, the activities of SOD, GPx, and CAT were restored, highlighting the effectiveness of ascorbate in enhancing antioxidant defenses. The histological examination of lung tissues in the group treated with ascrobate showed a reduction in inflammation and congestion when compared to the

exposed, untreated group. This provides more evidence for the beneficial effects of ascorbate on cypermethrin-induced lung injury. Similarly, the heart tissues showed enhanced architecture with decreased congestion, suggesting a protective function of ascorbate against cypermethrin-induced cardiotoxicity.

The results of this study aligned with those of Kaushik et al. (2018), who reported the protective effect of ascorbate on cypermethrin-induced organ damage. The group treated with alpha-tocopherol demonstrated notable improvements in both biochemical and histological parameters. Biochemically, decreased MDA levels in the lungs and heart indicated the attenuation of lipid peroxidation. The restoration of the enzymes SOD, GPx, and CAT further demonstrated the effectiveness of alpha-tocopherol in combating cypermethrin-induced oxidative stress. The histopathological analysis of lung tissues showed reduced inflammation and congestion. This finding corroborated the protective effects of alpha-tocopherol against cypermethrin-induced lung injury. Moreover, the heart tissues displayed strengthened architecture accompanied by a reduction in congestion, buttressing the cardio-protective role of alpha-tocopherol. It can be inferred that even though exposure to cypermethrin causes histopathological changes, the use of alpha-tocopherol helps to ameliorate the toxic effects (Abdus Sallam et al. 2020).

Interestingly, the co-administration of ascorbate and alpha-tocopherol demonstrated the most robust antioxidant effect among all experimental groups. The co-administration group displayed the lowest MDA levels, indicating the most effective mitigation of lipid peroxidation and oxidative damage. Additionally, this group exhibited elevated levels of SOD, GPx, and CAT activities, which suggest a synergistic antioxidant action of both ascorbate and alpha-tocopherol. Such synergistic action has also been documented in earlier research (Bhardwaj et al. 2018). The histological examination of the lungs in the treatment groups revealed mild peribronchiolar inflammatory reactions. However, the co-administration group showed a notable reduction in inflammation and a lack of congestion, suggesting enhanced protection against cypermethrin-induced lung injury. These results correspond with those of a previous study conducted by Oladele et al. (2022). The rats that received both vitamins exhibited the most favorable outcomes, according to the heart tissue examination. They showed a significant reduction in inflammation and congestion, underscoring the synergistic cardioprotective effects of both antioxidants.

Food contaminated with insecticides, such as cypermethrin, has an increased risk of harming both humans and domesticated animals. The prophylactic use of vitamins as a preventive measure in medical intervention for pathological conditions can be beneficial due to their ability to mitigate oxidative stress induced by cypermethrin exposure (Oladele et al. 2022, Akinpelu et al. 2023). Overall, this study underscores the detrimental effects of cypermethrin on the cardiopulmonary system. Therapeutic interventions using a combination of ascorbate and alpha-tocopherol effectively mitigate oxidative damage. The co-administration of both antioxidants provides the strongest protective effects (Pisoschi & Pop 2015, Huang et al. 2018). The biochemical and histological findings from this study support the potential use of ascorbate and alpha-tocopherol as protective agents against cypermethrin-induced oxidative stress. These substances were found to boost antioxidant defense mechanisms and enhance tissue architecture. This highlights the importance of antioxidant supplementation in combating oxidative stress induced by pesticides, thus warranting the need for more research to investigate potential clinical applications.

Strength and limitations

This study reflects the practical implications of cypermethrin exposure on local farmers in the Ondo community of Nigeria. It was done by simulating their exposure to the pesticide during farming activities through the use of animal models. The findings of this study provide evidence for the robust effect of ascorbate and alpha-tocopherol supplementation in combating pesticide-induced oxidative stress. However, the use of cypermethrin in this study merely represented a limited degree of the harmful effects of cypermethrin on the local farmers. The overall cytotoxicity of pyrethroids experienced by the local farmers might not be accurately reflected.

CONCLUSION

The administration of ascorbate and alpha-tocopherol provides protective effects against cypermethrin exposure by reducing oxidative damage. Furthermore, the co-administration of the vitamins has robust effectiveness. The combined use of these substances can boost the overall antioxidant capacity through a synergistic effect. Antioxidant supplementation is important in protecting against pesticide-induced oxidative stress. Further investigation is required to explore the potential clinical applications of ascorbate and alpha-tocopherol concerning cypermethrin exposure.

Acknowledgment

The authors would like to thank Dr. Gideon Oladipo of the Department of Biochemistry, Achievers University, Owo, Nigeria, for his expertise in biochemical analysis. Additionally, the authors would like to acknowledge MLS Seyi Otegbade of the Histopathology Unit of the University College Hospital, Ibadan, Nigeria, for processing the histology samples.

Conflict of interest

None.

Ethical consideration

The Research Ethics Committee (REC) of the University of Medical Sciences, Ondo, Nigeria, approved this study under reference No. NHREC/TR/UNIMED-HREC-Ondo St/22/06/21 on 3/6/2022. Appropriate measures were implemented to ensure minimal discomfort for the rats used in this study.

Funding disclosure

None.

Author contribution

AM conceptualized, designed, and drafted the article. AM analyzed and interpreted the data as well as performed a critical revision of the manuscript for important intellectual content. TDA granted final approval for the article. TDA provided the study materials. OFR collected and compiled the data. AM conducted the statistical analysis. TDA provided administrative, technical, and logistic support.

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Systematic Review

DEVELOPMENT OF DISCHARGE PLANNING FOR STROKE PATIENTS

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ABSTRACT

The annual incidence of stroke has continued to rise, establishing it as the leading cause of mortality and permanent disability globally. The quality improvement of discharge planning is essential throughout the development of discharge planning, which guarantees a seamless transition of care for stroke patients and family preparedness. This systematic review aimed to analyze the development of discharge planning and its impact on stroke patients and their families. The research was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Eligible pieces of literature were compiled from seven electronic databases, i.e., ScienceDirect, Scopus, PubMed, EBSCOhost, ProQuest, SAGE Journals, and Google Scholar. The literature search was performed using predetermined search terms, with specific criteria that included papers exclusively published in English and studies conducted in 2018–2022. This study included eleven eligible papers, from which we identified three distinct approaches to the development of discharge planning. Firstly, seven articles recommended a conventional approach, which involved specific educational intervention with interactive learning methods through booklets, textbooks, and PowerPoint presentations. Secondly, two articles supported the use of a technology-based approach through audiovisual media or applications to deliver health information. Thirdly, two articles endorsed a family-centered nursing approach that focused on empowering families to deliver health information to the patient. The development of discharge planning showed a significant impact on stroke patients, as it could influence various aspects of their quality of life. This included improvements in physiological function, enhanced cognitive knowledge, increased satisfaction and self-efficacy, reduced stress levels and care burden, and the opportunity for families to adequately prepare for home-based patient care. This study concluded that integrating conventional with technology-based media is effective for developing discharge plans for stroke patients. The implementation of this novel approach in a health system can improve patient outcomes, family preparedness when providing care, and the quality of hospital care.

Keywords: Discharge planning; stroke; health system and access; health risk

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Article history
●Submitted 27/1/2023 ● Revised 06/06/2023 ● Accepted 30/10/2023 ● Published 10/12/2023

How to cite: Mujib MIR, Arofiati F (2023). Development of Discharge Planning for Stroke Patients. Folia Medica Indonesiana 59 (4), 400-409, https://doi.org/10.20473/fmi.v59i4.42819

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

Highlights:
1. Given the frequent long-term complications of stroke, this study identified the ideal approach to discharge planning to improve the quality of life of patients and hospital care.
2. This study provides evidence supporting the effectiveness of a conventional discharge planning approach that incorporates specific educational intervention with interactive learning through audiovisual media.
3. The approaches presented in this study may offer valuable perspectives on enhancing health service provisions, particularly regarding the discharge planning process for nurses.

INTRODUCTION

Stroke is a public health issue that significantly affects the population of the world. Studies have shown that it has a high prevalence of morbidity and mortality rates (Whitehead & Baalbergen 2019, Chimatiro & Rhoda 2019). Currently, stroke is considered the second-leading cause of death on a global scale. Every year, 15 million people suffer from stroke and subsequently experience permanent disability (World Health Organization 2022, Temehy et al. 2022).

In addition to being physically impaired, stroke patients frequently experience cognitive impairments. These conditions further exacerbate their dependence on others for assistance (Westerlind et al. 2019, García-Pérez et al. 2021).

Discharge planning is crucial for overcoming these problems and improving treatment outcomes. It is imperative for health professionals to ensure the continuity of care and consistently provide training and education (Ngoc & Hsu 2021). The improvement of the health system through the development of discharge plans can effectively promote the seamless provision of medical services, thereby mitigating the risk of secondary strokes. This is in line with the third goal of the Sustainable Development Goals (SDGs), which is ensuring healthy lives and promoting well-being for all at all ages.

It is necessary to optimize the standard discharge plans that have been commonly used in recent times. This is because, according to a study, there is a significant prevalence of care discontinuity following the discharge of patients who have experienced a first-ever stroke (de Mooij et al. 2021). Studies have found that the implementation of discharge planning is frequently limited to the moment when patients leave the healthcare facility. Furthermore, the discourse surrounding discharge planning often centers around the evaluation of follow-up reporting cards (Asmuji et al. 2018, Soebagiyo et al. 2020).

Previous studies have primarily concentrated on the benefits and functions of discharge planning rather than on the development of a discharge planning model. In addition, there have been studies that focused on the general discussion of discharge planning findings in specific populations, such as stroke patients in Thailand (Simbolon et al. 2019, Suksatan & Posai 2020). As shown in the study by Lestari et al. (2020), limitations in implementing discharge planning will hinder adherence to care and follow-up visits due to the lack of information provided to patients and their families. It is clearly evident that further research is necessary to explore the development of discharge planning for stroke patients on a global scale. One approach that can be used is to conduct a thorough review of the available evidence derived from the findings of existing studies on discharge planning for stroke patients. Therefore, this systematic review aimed to analyze and synthesize the development of discharge planning used for the purpose of improving the recovery of stroke patients in hospitals.

MATERIALS AND METHODS

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, as outlined in the study conducted by Rethlefsen et al. (2021), were utilized to assist in the conduct of this systematic review. There were two research questions for this review: (1) What is the development approach of discharge plans for stroke patients? (2) What is the impact of such development on stroke patients and their families? The literature search was performed in seven electronic databases, i.e., Scopus, PubMed, ProQuest, ScienceDirect, EBSCOhost, SAGE Journals, and Google Scholar. The search terms were derived from the developed Medical Subject Headings (MeSH), which generated the following queries: ("Discharge planning" OR "Discharge, Patient" OR "Discharges, Patient" OR "Patient Discharges" OR "Planning, Discharge") AND (Stroke OR "Cerebrovascular Accidents" OR "CVA (Cerebrovascular Accident)" OR "Brain Vascular Accident" OR "Acute Cerebrovascular Accident" OR "Stroke patient"). All of the results of the literature search were exported to the Endnote software, version 20.0 (Clarivate, Philadelphia, PA, USA). We eliminated duplicates from the search results.

A reviewer (IR) initially screened each title and abstract of the scientific articles. Afterwards, an expert reviewer (FA) examined each one of the articles to ensure the compliance with the inclusion criteria. In the selection process, any disagreements that arose between the two reviewers were discussed and clarified with a third reviewer. The inclusion criteria were as follows: (1) hospital-based research examining discharge planning for stroke patients and their families; (2) studies employing a quantitative, qualitative, or mixed research method; (3) publications from the period of 2018–2022; and (4) articles written in English. Excluded from this study were review articles, protocol studies, and research pertaining to the development of home-based stroke interventions following discharge (Page et al. 2021).

The Joanna Briggs Institute (JBI) Manual for Evidence Synthesis, as described in the study conducted by Aromataris & Munn (2020), provided instructions for mapping the data in this study. Detailed evidence, characteristics, and research instruments were extracted from eleven selected studies. Each of the articles was examined multiple times by the reviewers (IR and FA) to ensure that all information was recorded correctly. Afterwards, a table was generated utilizing Microsoft Word for Windows, version 2021 (Microsoft Inc., Redmont, WA, USA) to map and organize the essential information. The categorization of the data obtained in this study is presented in Table 1. The results of the analysis of discharge planning development were categorized to identify the approach used in each of the selected studies. We summarized the relevant findings in a tabular format, with a categorization of the first author and publication year, study objectives, country, study design, population, sample size, interventions implemented

for discharge planning, identification of impacts on stroke patients and their families, and intervention procedure. Analysis using such data organization could help identify, analyze, and communicate patterns within the data sets.

RESULTS

The literature search yielded a total of 1,952 items, out of which 82 publications were found to be duplicates. Out of the remaining 1,870 items, the title and abstract of each publication were carefully scrutinized according to the predetermined inclusion criteria. A total of 123 full-text papers were independently screened to determine the suitability of the research. The flow diagram depicted in Figure 1 illustrates the process of conducting a literature search in accordance with the PRISMA guidelines to discover the eligible papers for this study.

At the conclusion of the literature search, eleven scientific articles were found to be eligible. Table 1 presents the characteristics of the studies included in this systematic review. The majority of the selected articles were published in 2019. The included articles were written in seven countries: Indonesia (n=4), Iran (n=2), Egypt (n=1), America (n=1), France (n=1), Australia (n=1), and the Netherlands (n=1). The total number of participants in all of the studies was 1,610, consisting of 1,333 stroke patients and 277 family members or caregivers. The selected articles mostly reported on quantitative studies, including randomized control trials (RCT) (n=5), quasi-experimental studies (n=4), cross-sectional studies (n=1), and mixed-method studies (n=1).

The reviewed articles were categorized according to their approaches to discharge planning for stroke patients. The discharge plans were developed with an emphasis on various factors. A conventional approach with specific educational interventions was employed in the studies conducted by Simbolon et al. (2019), Iskandar et al. (2018), Taha & Ibrahim (2020), Boden-Albala et al. (2019), Benoit et al. (2020), Andrew et al. (2018), and Amiri et al. (2022). The conventional approach entailed a development model to directly address the needs of patients and caregivers. This was achieved by providing additional training and educational programs that were interactive and organized, utilizing media such as booklets, textbooks, or PowerPoint presentations. On the other hand, the studies by Kurniati et al. (2022) and Vloothuis et al. (2019) utilized information technology in developing discharge planning. The incorporation of information technology in the discharge planning process involved the use of audiovisual learning programs, a combination of PowerPoint presentations and educational videos, and e-health applications such as caregiver-mediated exercises with e-health support for early supported discharge after stroke (CARE4STROKE). The two other studies conducted by Mohammadi et al. (2019) and Dharma et al. (2021) focused on the development of discharge planning that incorporated family-centered nursing. The importance of families and caregivers was highlighted by supporting their empowerment to provided improved assistance and care for stroke patients.

Among the seven articles that discussed conventional approaches with specific educational interventions, two articles revealed the beneficial impact of the intervention on physiological changes in stroke patients. Specifically, these studies observed an improvement in muscle strength on day 7 and a reduction in blood pressure at a 12-month follow-up duration (Iskandar et al. 2018, Boden-Albala et al. 2019). The other studies revealed various beneficial effects of well-developed discharge planning. Some of the benefits were the increased independence and patient satisfaction during hospital treatment, improved performance in daily living activities, a higher quality of life, enhanced knowledge on stroke and risk factors, strengthened self-efficacy, and a reduction in unmet needs (Andrew et al. 2018, Simbolon et al. 2019, Taha & Ibrahim 2020, Benoit et al. 2020, Amiri et al. 2022).

There were two studies by Vloothuis et al. (2019) and Kurniati et al. (2022) that supported the utilization of technology, and both articles presented evidence regarding the benefits of the intervention. The incorporation of technology in the development of discharge planning demonstrated the potential to reduce anxiety and depression in stroke patients' families. It could also improve family preparedness to provide care for stroke patients at home. Meanwhile, the development of discharge planning using a family-centered nursing approach in the other two studies by Dharma et al. (2021) and Mohammadi et al. (2019) demonstrated further benefits, including the improvement of adaptive coping skills in families and the reduction of family burden. It was also revealed that there were increased levels of family preparedness and reduced stress in stroke patients' families.

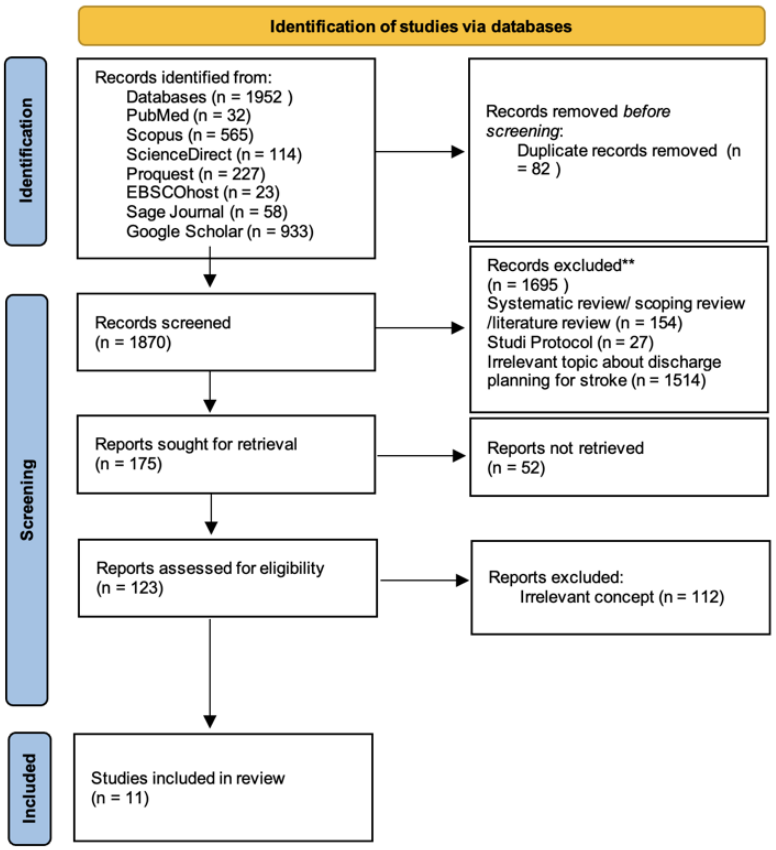

Figure1. PRISMA flow diagram of the literature search process.

Table 1. Summary of the selected article

Author/year	Development of discharge planning	Study design, samples, country, and instruments	Findings	Discharge planning intervention procedure
Simbolon et al. (2019)	Discharge planning model for stroke patients with hypertension vs. standard discharge planning.	Design: Quasy-experimental Sample: 160 stroke patients Country: Indonesia Instruments: -	A significant influence on independence and satisfaction after the provision of a discharge planning with stroke education.	Media: Training module. Time: From admission until discharge. Materials: Treatment of stroke patients with hypertension.
Iskandar et al. (2018)	Discharge planning that incorporated ROM training vs. standard discharge planning.	Design: Quasy-experimental Sample: 34 stroke patients Country: Indonesia Instruments: -	A significant increase in muscle strength for both the upper and lower extremities in the treatment group (on day 7) and control group (on day 14).	Media: ROM monitoring sheet. Time: From admission up to 14 days of treatment. Materials: Stroke-related information and ROM exercises.

Author/year	Development of discharge planning	Study design, samples, country, and instruments	Findings	Discharge planning intervention procedure
Taha & Ibrahim (2020)	A discharge planning program with an evaluation of the quality of life and daily living activity of stroke patients.	Design: Quasy-experimental Sample: 50 stroke patients Country: Egypt Instruments: Structured interview questionnaires, i.e., Stroke Specific Quality of Life Scale (SS-QOL) and Barthel Index for Activities of Daily Living (ADL).	An increase in the mean score of stroke knowledge, daily living activity, and quality of life after the implementation of discharge plans.	Media: Textbooks with concise wording and illustrations. Time: Four sessions with a duration of 45–60 minutes each. Materials: • First session: Theoretical knowledge regarding the definition, signs, symptoms, risk factors, medications, complications, and recurrence prevention of stroke. • Second session: Knowledge related to daily living activities. • Third session: Instructions for patients regarding exercise, dietary restriction, hydration, personal hygiene, adaptive clothing, medications and aids, and a healthy lifestyle. • Fourth session: Physical activity recommendations to improve physical health.
Boden-Albala et al. (2019)	Discharge education strategy vs. standard discharge care.	Design: RCT Sample: 552 stroke patients Country: USA Instruments: -	A statistically significant decrease in systolic average blood pressure of 9.9 mmHg among Hispanic individuals after the one-year follow-up duration, compared to regular. treatments	Media: PowerPoint presentations, books, and videos. Time: Before discharge and during follow-up with durations at 72 hours and 1, 3, 6, and 12 months. Materials: Patient-physician communication, medication adherence, risk mitigation skills, and motivations for behavior change.
Benoit et al. (2020)	Pre-discharge educational intervention vs. standard discharge care.	Design: RCT Sample: 199 stroke patients Country: France Instrument: stroke knowledge score (SKS)	A significant improvement in stroke knowledge score and increased knowledge of risk factors.	Media: PowerPoint presentations. Time: Two-hour session before discharge. Materials: Knowledge related to the mechanisms, risk factors, warning symptoms, and emergency aid of stroke to ensure patient comprehension through an interactive learning process.
Amiri et al. (2022)	Self-management program vs. standard care.	Design: RCT Sample: 72 stroke patients Country: Iran Instrument: The Jones Stroke Self-Efficacy Questionnaire	Significant changes in mean self-efficacy scores immediately after the intervention and three months later.	Media: Booklets on stroke training and post-discharge home care. Time: 6 weeks. Materials: Training on treatment status, medication management, symptom management (e.g., sleep management, relaxation, and fatigue management), psychological component management (e.g., self-regulation, emotional control, and anger, depression, stress, and anxiety management), lifestyle (exercise, diet, nutrition, and smoking restriction), social support (e.g., family communication and assistance), effective communication (e.g., communication strategies), problem-solving, and decision-making skills.

Author/year	Development of discharge planning	Study design, samples, country, and instruments	Findings	Discharge planning intervention procedure
Andrew et al. (2018)	Standard discharge planning.	Design: Cross-sectional study Sample: 200 stroke patients Country: Australia Instruments: EQ-5D-3L and VAS	An improvement in patients' quality of life and a reduction in unmet needs after discharge.	Media/time/materials: - Additional information: A simple strategy to improve the quality and flexibility of stroke-specific discharge planning, which can provide information regarding referrals to group rehabilitation and local support services according to patients' needs.
Kurniati et al. (2022)	Discharge planning that combined audiovisual media and family-centered nursing vs. standard discharge planning.	Design: Mix-method Sample: 71 family members of stroke patients Country: Indonesia Instruments: -	Phase 1: Knowledge regarding the implementation of discharge planning, specifically focusing on discharge time, treatment, and follow-up appointments. Phase 2: Increased family preparedness in caring for post-acute stroke patients before discharge	Media: Audiovisual media. Time: Three sessions, i.e., at admission, during treatment, and before discharge. Materials: Treatment of stroke patients.
Mohammadi et al. (2019)	Family- or caregiver-oriented discharge planning program vs. standard discharge planning.	Design: RCT Sample: 60 family members of stroke patient. Country: Iran. Instrument: The Kingston Caregiver Stress Scale (KCSS) and the Preparedness for Caregiving Scale (PCS).	A significant increase in family preparedness levels and lower stress compared to the control group.	Media: - Time: Three sessions with a duration of 60–120 minutes each. Material: Information regarding the causes and risk factors of stroke, the importance of treatment, the role of relatives in stroke care, cognitive and emotional effects, sensory complications (e.g., attention deficit, awareness impairment, depression, visual disturbance, and disorientation), movement disorders (e.g., paralysis), bowel and bladder dysfunction, sleep problems (e.g., excessive daytime sleepiness), urinary tract infections, and pneumonia.
Dharma et al. (2021)	The Caregiver Empowerment Program Based on the Adaptation Model (CEP-BAM) vs. conventional intervention through discharge planning programs for caregivers.	Design: Quasy-experimental Sample: 80 family members of stroke patients. Country: Indonesia Instrument: The Caregiver Coping Questionnaire and the Caregiver Burden Scale (CBS).	An improvement in coping ability at 5–6 months and a decrease in family burden at 4-6 months after the intervention.	Media: CEP-BAM intervention module. Time: Three sessions with a duration of 60–120 minutes each, scheduled over a two-week period. Materials: (1) Stroke information for caregivers, i.e., the challenges and experiences of stroke patients as well as the prevention of recurrent strokes; (2) Training for caregivers to perform adaptive coping strategies, assist physical adaptation (ambulation), aid patients in walking exercises and joint movements, and help patients with daily activities such as bathing, using the restroom, getting dressed, and practicing eating; (3) Education for caregivers on providing emotional support to patients and maintaining their own mental well-being while caring for patients.

Author/year	Development of discharge planning	Study design, samples, country, and instruments	Findings	Discharge planning intervention procedure
Vloothuis et al. (2019)	Caregiver-mediated exercises with e-health support for early supported discharge after stroke (CARE4STROKE) vs. standard care.	Design: RCT Sample: 66 stroke patients and 66 caregivers Country: Netherlands Instruments: For patients: SIS 3.0 and length of stay . For caregivers: The Caregiver Strain Index and the Care Quality of Life Scale.	• Significant decrease in caregiver depression and patient anxiety after the intervention, which remained consistent until the week 12 follow-up. appointment • No significant improvements in the length of stay or the mobility component of the SIS after 8 and 12 weeks.	Media: CARE4STROKE e-health application. Time: 150-minute session per week over the course of eight weeks. Material: 37 options for standard exercises to improve mobility.

Notes: RCT=Randomized control trial; ROM=Range of motion; EQ-5D-3L=The 3-level European Quality of Life-5 Dimensions; VAS=Visual Analogue Scale; SIS 3.0=The Stroke Impact Scale 3.0; the length of stay refers to the time between the onset of a stroke and the discharge from the rehabilitation facility.

DISCUSSION

This study reviewed the evidence of existing studies on the development of discharge plans for stroke patients and their families. Furthermore, the purpose of this systematic review was to outline the approaches used to develop discharge plans as well as their effects on stroke patients and their families or caregivers. Duangchan et al. (2022) described that the implementation of discharge planning generally consists of four phases: patient assessment, plan development, provision of plans, and follow-up evaluation. However, the components of discharge planning vary between countries due to differences in healthcare systems, cultural attitudes, and patient needs.

The studies reviewed were conducted in seven countries and employed three different approaches in developing discharge plans. The first approach was a conventional method that incorporated specific educational interventions. Benoit et al. (2020) emphasized that educational interventions in discharge planning are elementary, realistic, and easy to implement in a stroke unit. Educational interventions implemented by nurses were found to have more impactful outcomes in stroke patients compared to passive methods such as providing video content, booklets, or multimedia applications. The dissemination of information via technology, such as videos, leaflets, guidebooks, or multimedia applications, resulted in patients needing further clarification and facing difficulties in directly posing interactive questions to healthcare professionals.

The second approach relied on the utilization of technology, namely CARE4STROKE, to enhance the emotional well-being of caregivers. This approach was implemented by providing them with meditation exercises aimed at reducing their stress levels. They were reported to experience enhanced engagement in the rehabilitation process and improved preparedness for home-based care (Vloothuis et al. 2019). The utilization of technology in the form of audiovisual media in the study conducted by Kurniati et al. (2022) provided families with retrievable and replayable information that might be easily accessed at home in case they forgot specific details. This aligns with previous studies that found video instruction effectively delivered concise information, regardless of the patients' health literacy or ability to communicate with healthcare professionals. The implementation of e-discharge planning for heart failure patients demonstrated the potential to affect self-care and medication management, increase knowledge, and facilitate remote patient monitoring for healthcare professionals (Jové-Blanco et al. 2021, Aryadi & Arofiati 2021).

During the literature search for this systematic review, we discovered numerous protocol studies that introduced innovative approaches to technology-based discharge planning for stroke patients. One of the studies was a research project with discharge planning that utilized machine learning (Bacchi et al. 2022). Previous studies have

documented the utilization of online information in inpatient units specifically catering to individuals suffering from stroke. The applications of trees in data structure accelerated the development of discharge plans for stroke patients through the efficient organization and optimization of data storage and retrieval (Archambault et al. 2020, Veerbeek et al. 2022). In a separate study, Wang et al. (2022) employed a protocol to build an online clinical practice guide specifically designed for the discharge planning of stroke patients. However, further evaluation is required to ascertain the impact of these technologies on stroke patients and their families.

The third approach emphasized family-centered nursing, with all training and educational activities specifically tailored to address the needs and concerns of families. Rodakowski et al. (2017) conducted prior research on the involvement of caregivers in the discharge planning process for older adults. The study revealed that implementing discharge planning interventions that involve caregivers' participation could effectively reduce the risk of hospital readmissions. Mohammadi et al. (2019) reported that the provision of support programs in discharge planning to family members of stroke survivors, which included training in adult learning theory, was able to improve care. The effectiveness of the program resulted from the families' attentive participation in all educational and learning sessions.

Upon reviewing eleven articles, we discovered that the implementation of discharge planning for stroke patients resulted in favorable outcomes. These included improvements in quality of life, stroke-related knowledge, patient independence, and family preparedness to care for stroke patients. The conventional approach, involving direct and active educational instruction by nurses, was the most commonly employed method in the development of discharge planning. Media support and educational content help nurses provide health education during the discharge planning process. The studies conducted by Iskandar et al. (2018) and Boden-Albala et al. (2019) focused on educational interventions for stroke patients during discharge planning. These interventions specifically addressed the need for range of motion (ROM) training and education on hypertension for stroke patients. Further studies are necessary to address the different discharge requirements of each patient. The precise interventions implemented in the discharge planning process can assist patients in fulfilling their requirements during the discharge preparation process.

The utilization of educational media in the discharge planning process was shown to be most efficient when incorporating PowerPoint presentations or video media featuring illustrated, animated, or photographic information. This can facilitate patients' comprehension of the material being presented. The research conducted by Kurniati et al. (2022) and Vloothuis et al. (2019) highlighted that implementing a technology-based information system and utilizing audiovisual or video-based educational materials in discharge planning can enhance the preparedness of families to provide care for stroke patients. Audiovisual media featuring animations, infographics, or photographic images can improve one's comprehension of a topic by providing contextual convenience. Discharge planning utilizing media, modules, or booklets remained prevalent in numerous studies. However, brochures have limitations relating to visualization, narrow space and context, minimal interaction, and constrained accessibility. Furthermore, the absence of interesting auditory and visual elements contributes to a deficiency in emotional engagement, which may result in learners being less actively immersed in the learning process. In practical application, a combination of modules or brochures accompanied by audiovisual media offers a higher level of accessibility for patients and their families to acquire comprehension of discharge preparations (Boden-Albala et al. 2019, Benoit et al. 2020).

It is imperative that the development of discharge planning include the active involvement of families in every care process to ensure the seamless continuation of care for stroke patients upon their return home. Dharma et al. (2021) described that employing a family-centered nursing approach offers the benefit of improving families' adaptive coping skills and equipping them with the capacity to use the skills when faced with challenges. By using these coping skills, caregivers can effectively reduce their burden, manage stress levels, and maintain an ideal quality of life. The provision of care, attention, and affection by the family to ill family members will aid in the patients' physical and psychological recovery (Hutagalung 2021).

Strength and limitations

This systematic review provides a comprehensive overview of the latest developments in discharge planning for stroke patients. It includes a detailed explanation of the methods employed in the development of discharge planning, the mechanism for intervention procedures, and the outcomes observed in stroke patients and their families. However, we encountered challenges in collecting articles that specifically addressed the topic of discharge plans for stroke patients. Not all articles on discharge planning for stroke patients in various countries were written in English.

CONCLUSION

The conventional approach, with the incorporation of specific educational interventions, is the most widely used method in the development of discharge planning for stroke patients. A conventional approach combined with interactive learning through audiovisual media appears to be effective in developing discharge planning to improve patient outcomes and equip families to be prepared to take care of their sick family members. Clear and structured educational interventions that actively involve nurses in the discharge planning process provide more convincing evidence of patient comprehension. Further research is required to evaluate the effectiveness of other alternative approaches in discharge planning for stroke patients and their families or caregivers.

Acknowledgment

The authors would like to thank those who contributed to the completion of this research, including the Nursing Postgraduate Program at Universitas Muhammadiyah Yogyakarta, Bantul, Indonesia. Additionally, the authors extend gratitude to the reviewers who provided constructive criticism.

Conflict of interest

None.

Funding disclosure

None.

Author contribution

MIR contributed to the conception and design, analysis and interpretation of the data, drafting of the article, and collection and assembly of the data. FA contributed to the critical revision of the article for important intellectualcontent, final approval of the article, and administrative, technical, and logistic support.

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Systematic Review

THROMBOCYTOPENIA AS A CLINICAL BIOMARKER OF RETINOPATHY OF PREMATURITY

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ABSTRACT

Retinopathy of prematurity (ROP) is the primary cause of childhood blindness. It arises from the underdevelopment of retinal blood vessels in premature infants. Platelets have a vital function in the regulation of angiogenesis. Thus, thrombocytopenia may contribute to the progression of ROP. The objective of this systematic study was to examine the relationship between thrombocytopenia and ROP. The PubMed and Cochrane Library databases were accessed to search for retrospective, case-control, and cross-sectional studies. This study adhered to the guidelines outlined by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). The literature search resulted in nine records to be analyzed in our review. All the selected studies were conducted between 2017 and 2022. Seven studies reported that the prevalence of thrombocytopenia in infants with ROP ranged from 18.37% to 71%. The frequency of thrombocytopenia in preterm children without ROP was between 5.71% and 21%. Thrombocytopenia was identified as a risk factor for ROP in seven studies, with the odds ratio (OR) for thrombocytopenia ranging from 2.8 to 6.69. Thrombocytopenia in premature infants can potentially serve as a clinical biomarker in the screening of type 1 ROP. This finding suggests that thrombocytopenia may contribute to the pathophysiology of ROP. Further research is necessary to determine the critical threshold platelet count for thrombocytopenia in infants with ROP.

Keywords: Thrombocytopenia; retinopathy of prematurity (ROP); preterm infant; angiogenesis; childbirth complications

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Article history
●Submitted 28/1/2023 ● Revised 27/9/2023 ● Accepted 12/11/2023 ● Published 10/12/2023

How to cite: Setyawan IWE, Surasmiati NMA, Agrasidi PA, et al (2023). Thrombocytopenia as a Clinical Biomarker of Retinopathy of Prematurity. Folia Medica Indonesiana 59(4), 410-417, https://doi.org/10.20473/fmi.v59i4.42970

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

Highlights:
1. This is the first systematic review investigating thrombocytopenia and its association with retinopathy of prematurity.
2. Thrombocytopenia can be a useful clinical biomarker in retinopathy of prematurity screening, considering the quick, affordable, and widespread availability of the examination.

INTRODUCTION

Retinopathy of prematurity (ROP) is a proliferative condition that affects the vasculature of the retina in premature newborns. It has become the predominant cause of visual impairment in children globally (Kim et al. 2018). Around the world, ROP has caused at least 50,000 children to go blind. The blindness brought on by ROP occurs infrequently in developed countries. However, the occurrence of ROP leading to blindness is more common in developing countries due to a higher survival rate among premature infants, inadequate regulation of the neonatal intensive care unit (NICU), and scarce funduscopic examinations. These factors limit the frequency and accessibility of monitoring observable changes in the retinal vasculature (Wood et al. 2021).

Low gestational age and low birth weight are the primary factors that increase the risk of ROP development. According to a study by Hong et al. (2022), underdeveloped retinal vasculature and neurons are correlated with both low gestational age and birth weight due to the delicate retinal structure during birth. However, the study did not find a statistically significant relationship between birth weight and severe ROP. This finding might point to the significance of weight increase in slowing the course of ROP. Other risk factors include gender, the

need for oxygen therapy and blood transfusions, as well as the presence of patent ductus arteriosus (PDA), intraventricular extension of hemorrhage, necrotizing enterocolitis (NEC), sepsis, and newborn infections.

Elevated levels of oxygen pressure in ROP inhibit the formation of new blood vessels. As a result, the function of existing retinal capillaries will be impeded, causing non-vascularized regions within the retina. When the retina experiences hypoxia due to incomplete vascularization, it stimulates the secretion of angiogenic factors, such as vascular endothelial growth factor (VEGF) and erythropoietin. These factors contribute to the development of neovascularization, intraocular fibrosis, and retinal detachment. VEGF and vascular permeability in the eye have a significant role in pathological neovascularization. Anti-VEGF medication has been utilized to treat ROP due to the well-established link between vascular endothelial growth factor and ROP development (Eldweik & Mantagos 2016, Wu & Wu 2017).

Thrombocytopenia can be quickly and efficiently identified using standard blood tests. Additionally, the platelet transfusion procedure is a commonly used treatment (Sola-Visner & Bercovitz 2016). During the development of ROP, understanding the etiology of thrombocytopenia in ROP may be beneficial for the screening and treatment of the disease. A recent study found a correlation between thrombocytopenia and a worse prognosis for preterm newborns with neonatal sepsis and NEC (Resch et al. 2018). This systematic review aimed to analyze the relationship between thrombocytopenia and ROP.

MATERIALS AND METHODS

Previous research conducted by Page et al. (2021) provided a reference for the literature search strategy in this study. This systematic review was carried out by adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The Cochrane Library and PubMed databases were used in the comprehensive literature search in this systematic review. The search was performed to gather scholarly records of research conducted between September 2012 and September 2022. The following keywords and Boolean operators were used to search for the literature: (“Thrombocytopenia” OR “Low Thrombocyte” OR “Thrombocyte” OR “Platelet”), AND (“ROP” OR “Retinopathy of Prematurity").

We included all pertinent retrospective, case-control, and cross-sectional studies on thrombocytopenia in premature infants with ROP. Case reports, letters, editorials, review articles, inaccessible full texts, and studies in languages other than English were excluded from consideration (Pollock & Berge 2018). The flow diagram that summarizes the study selection process is exhibited in Figure 1.

Data were extracted from all of the selected studies. The general information (e.g., author and year of publication), participants, average gestational age, average birthweight, platelet count threshold for thrombocytopenia, prevalence of thrombocytopenia in infants with ROP, and the relationship between thrombocytopenia and ROP were among the collected data (Lee et al. 2017).

A checklist from the Joanna Briggs Institute was used to assess the quality and bias risk of the studies. Each item on the checklist was counted for one point. If a study received at least half of the maximum possible points, it was considered to be of high quality. If it scored fewer than half of the maximum possible points, it was deemed low quality (Lockwood & Oh 2017). Two reviewers independently assessed the quality of the studies to prevent bias. Any disagreement between the two reviewers was settled by consensus.

RESULTS

In our initial search, we discovered 117 scholarly records. Out of the 117 records, only nine original research papers were eligible for inclusion in this study. Figure 1 displays the flow diagram that demonstrates the process of the study selection. All of the studies met the high-quality standards set by the Joanna Briggs Institute. All of the selected studies included retrospective, cross-sectional, and case-control investigations. The sample sizes ranged from 9 to 240 individuals. Among the samples were infants born with multiple gestations, type 1 ROP, aggressive posterior retinopathy of prematurity (APROP), very low birth weight (VLBW), and prematurity. The mean birthweight of the newborns diagnosed with ROP varied between 585 and 1,955 grams. The infants diagnosed with ROP had a mean gestational age between 24 and 33.25 weeks. The summaries of the studies are presented in Table 1.

The platelet count threshold for thrombocytopenia exhibited variability across different studies. Among the nine studies examined in this systematic review, three used a threshold of 150,000/mL. Two studies specified a threshold of 100,000/mL. However, the remaining four studies did not specify the threshold for low thrombocyte levels.

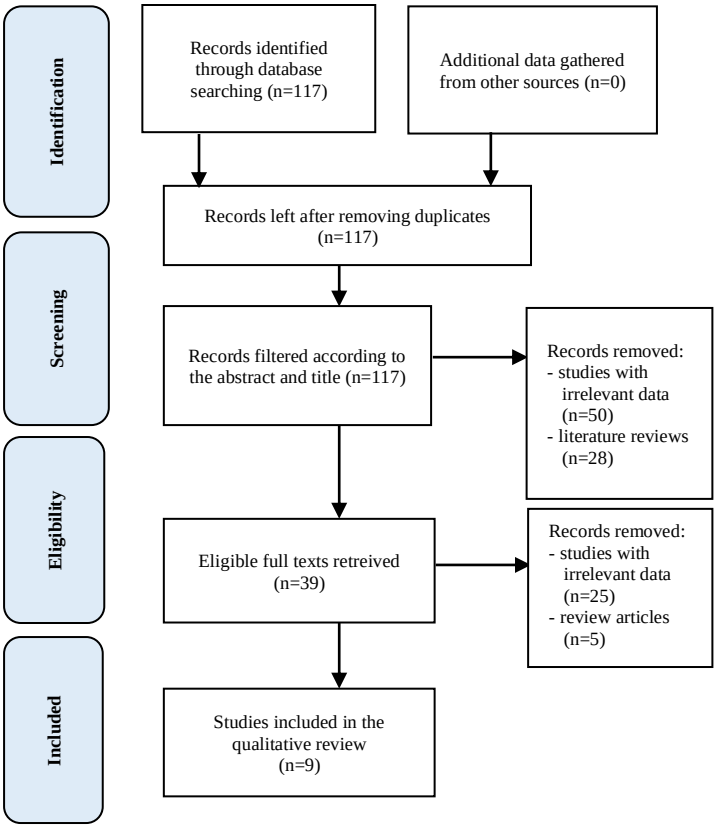

Figure 1. Flow diagram for the study selection process.

Table 1. Summaries of the characteristics of the included studies.

No	References	Research design	Total samples	Mean gestational age (weeks)	Mean birth weight (g)	Low thrombocyte level threshold	Thrombocytopenia prevalence (%)		Relationship between ROP and low thrombocyte levels
							Cases	Controls	
1	Sancak et al. (2019)	Case-control	81 infants with type 1 ROP and 81 premature infants without ROP	27.6±2.1	993±292	<150,000	71	21	RR=6.69; p<0.001
2	Cakir et al. (2018)	Retrospective	202 infants with VLBW	25.3±1	782±167	<100,000			OR=4.17; p<0.001 OR=2.97; p=0.006
3	Jensen et al. (2018)	Case-control	100 infants with type 1 ROP and 100 premature infants without ROP			<150,000			Weeks 24-28: OR=4.7; p=0.001 Weeks 29-34: OR=2.8; p=0.006 Weeks 35-38: OR=2.0; p=0.10 (not

									significant)
4	Lundgren et al. (2017)	Retrospective	9 infants with APROP and 9 infants with stadium II ROP	24±1	585 (470-700)	<100,000	56	27	Significantly lower thrombocyte counts (p<0.001) and a shorter time interval (in days) for platelet transfusion (p<0.001) among infants with APROP
5	Parrozzani et al. (2021)	Retrospective	206 infants with ROP and 357 premature infants without ROP	28.72±2.58	1083.8±329.62	-	18.37	5.71	Higher rates of low thrombocyte count among the cases (p=0.0071)
6	Choręziak et al. (2019)	Retrospective	76 infants receiving ROP management and 87 patients with ROP spontaneous regression	25±1.72	830±206	-	39.5	32.2	Higher rates of low thrombocyte count among the cases (p=0.015)
7	Özkaya (2022)	Retrospective	40 infants with type 1 ROP and 40 premature infants without ROP	26.5 (24–32)	925 (430–1440)	<150,000	25	10	Non-significant difference in the platelet counts between infants with ROP and premature infants without ROP (p=0.094)
8	Gaber et al. (2021)	Retrospective cross-sectional	240 premature infants	33.25±2.74	1955.23±692.43	-			Low thrombocyte counts as a risk factor for ROP (OR=2.0 (0.6-6.5))
9	Yau et al. (2015)	Retrospective cross-sectional	153 multiple-gestation infants	30.8±2.4	1284.8±267.4	-			OR=1.76; p=0.57 (not significant)

Five studies conducted by Sancak et al. (2019), Lundgren et al. (2017), Parrozzani et al. (2021), Choręziak et al. (2019), and Özkaya (2022) have documented low thrombocyte counts in infants diagnosed with ROP. The prevalence of thrombocytopenia among the research subjects ranged from 18.37% to 71%. These results indicated that preterm infants without ROP might also have decreased thrombocyte counts, affecting approximately 5.71% to 21% of the cases.

Seven studies conducted by Sancak et al. (2019), Cakir et al. (2018), Jensen et al. (2018), Gaber et al. (2021), Yau et al. (2015), Parrozzani et al. (2021), and Lundgren et al. (2017) have identified low thrombocyte counts as a significant risk factor for ROP. The range of the odds ratio (OR) for thrombocytopenia was found to be between 2.8 and 6.69. Most of these studies compared the occurrence of low thrombocyte counts between premature infants with type 1 ROP and premature newborns without ROP. The results of the studies revealed a significant relationship between the two conditions. However, thrombocytopenia did not appear to have any discernible effect on ROP, according to the analysis of zone 2 ROP. This was specifically apparent in newborns with a gestational age between 35 and 38 weeks, as well as between infants with stabilized ROP and those with progressive ROP.

DISCUSSION

ROP is a complex vasoproliferative condition affecting the retinas of premature infants born with inadequate vascularization. It has emerged as a significant newborn illness that causes about 40% of all childhood blindness worldwide. Advancements in neonatal care have led to an increase in the survival rate of infants with low birth weight, thereby increasing the incidence of ROP (Tan et al. 2022).

According to the comprehensive review in this study, thrombocytopenia increases the probability of ROP development in premature infants. However, the specific mechanism by which thrombocytopenia impacts the progression of ROP and the balance of VEGF remains unknown. Existing studies have highlighted the crucial regulatory function of angiogenic factors, such as insulin-like growth factor 1 (IGF-1) and VEGF. Platelets transport, store, and release both of these angiogenic factors (Sancak et al. 2019, Dai et al. 2021, Guo et al. 2021).

There are two phases in the pathogenesis of ROP. High oxygen levels in the uterus during the first phase decrease retinal VEGF expression. This mechanism prevents the interference of blood in the growth of blood vessels, which results in the constriction of retinal capillaries and the formation of avascular regions in the retina. During the second phase, the infant experiences relative hypoxia, which subsequently triggers the upregulation of VEGF by Müller cells and astrocytes. The surge in VEGF expression results in neovascularization in the retina, which is a pathognomonic feature of ROP (Guo et al. 2021).

Proliferative retinopathy has been associated with increased production of endogenous IGF-1, which is a consequence of retinal VEGF accumulation during the second phase of ROP pathogenesis. Therefore, thrombocytopenia may contribute to insufficient VEGF sequestration in the developing retina, subsequently leading to the onset or progression of proliferative retinopathy. Examinations of platelet counts and the related factors are relevant in both the first and second phases of ROP. Numerous studies investigating ROP pathogenesis in the first phase did not uncover significant differences in thrombocyte parameters. Therefore, it might indicate that platelets exhibited heightened activity. Meanwhile, in the second phase, the platelets could induce a rise in neovascularization. Certain studies among the chosen literature only included the second phase, while other studies included both the first and second phases. However, the precise function of platelets in regulating VEGF has not been well understood (Cakir et al. 2018).

The impact of thrombocytopenia on APROP has been investigated previously. A large discrepancy was observed in the platelet counts between infants diagnosed with APROP and those without the disease. Platelet transfusions helped alleviate thrombocytopenia in a severely ill patient experiencing spontaneous regression of APROP by increasing the platelet count from 21,000/mm^3 to 118,000/mm^3. In a separate setting with 21 controls and 9 APROP cases, there was a significant difference in thrombocyte counts between patients with APROP and the control participants. Low platelet counts can hinder the ability of preterm children with APROP to effectively regulate the excessive production of VEGF caused by peripheral retinal ischemia and maintain optimal VEGF levels (Seliniotaki et al. 2022).

Platelet transfusions were found to prevent vascularization in mouse animal models of oxygen-induced retinopathy. Mice affected by retinopathy had lower platelet counts compared to those grown in normal conditions. Platelet depletion in mouse models of ROP resulted in an increase in the formation of abnormal blood vessels in the retina, known as neovascular tufts. Conversely, platelet transfusion exerted the opposite effect by decreasing the formation of neovascular tufts. A statistically significant difference was observed in the average

weekly platelet counts of the mice, distinguishing those with severe ROP from those with no or milder ROP (Cakir et al. 2018).

Thrombocytopenia is a common hematologic condition in newborns that can cause death and morbidity. It affects approximately 1-5% of all newborns, 12% of preterm infants, and 20–40% of infants receiving intensive care. Thrombocytopenia becomes more common as gestational age and body weight decrease. A decrease in thrombocyte count can contribute to the development of ROP by disrupting the optimum balance of angiogenesis-regulating substances, such as VEGF, endostatin, and thromboxane A2. The results of this study aligned with recent research, which observed a correlation between severe ROP and thrombocytopenia (Şahinoğlu Keşkek et al. 2020, Seliniotaki et al. 2022).

Several studies conducted by Cakir et al. (2018), Sancak et al. (2019), and Kumawat et al. (2021) have found that thrombocytes have an anti-angiogenic effect on the formation of the retina. This occurs due to the removal of excess VEGF during the vascularization stage. Thus, thrombocytopenia is associated with a more severe form of ROP. In addition, there is a correlation between thrombocytopenia and low levels of VEGF-A (Lundgren et al. 2017).

Platelets are recognized as having a storage and transportation system for various pro- and anti-angiogenic regulators. The regulators include insulin-like growth factor binding protein (IGFBP-3), which serves as the primary serum binding protein for IGF-1, VEGF, and platelet-derived growth factor (PDGF). Each of these regulators is present within the alpha granules of platelets. It was observed that severe thrombocytopenia improved significantly following the administration of serum platelet transfusions to a patient with APROP. Furthermore, a correlation was discovered between thrombocytopenia and type 1 ROP in zone 1. These findings suggest that thrombocytopenia can be considered a risk factor for zone 1 ROP (Holinstat 2017, Cakir et al. 2018).

Platelets contain three distinct types of secretory granules that store bioactive molecules. These components include dense granules, alpha-granules, and lysosomes. Among these granules, the most common ones are angiostatin, platelet factor 4, thrombospondin 1, 2-macroglobulin, endostatin, and plasminogen activator inhibitor 1. Although platelets contain both pro- and anti-angiogenic substances, previous studies have mostly focused on investigating the pro-angiogenic or pro-proliferative properties of platelets when analyzing their effects on endothelial cells (Rubio & Adamis 2016, Sharda & Flaumenhaft 2018).

A study discovered a correlation between severe ROP and thrombocytopenia occurring from birth to a postmenstrual age (PMA) of 34 weeks. None of the newborns in the study required platelet transfusions, and their platelet counts did not decrease to levels that indicate thrombocytopenia. However, significant differences in platelet counts were observed between infants who developed ROP and those who did not. Platelets have a pivotal role in regulating angiogenic factors, such as VEGF and PDGF. The regulation is possible through the storage, transportation, and release of these factors. Therefore, platelets can serve as crucial regulators for the aforementioned proteins (Jensen et al. 2018, Özkaya 2022).

Strength and limitations

The main strength of this study was that it was regarded as the first comprehensive review of the relationship between ROP and thrombocytopenia. In contrast to previous studies that included only a limited scope of research, this study incorporated a wider range of research. However, it is important to take into account a few drawbacks of this study. Two of the studies included in this systematic review were cross-sectional, while the rest were retrospective. This might affect the reliability and validity of the evidence presented. The study populations were diverse, while thrombocytopenia is characterized by a certain threshold of platelet count that can differ across individuals. Additionally, different centers might have used various instruments to evaluate platelets, potentially leading to variations in diagnosis accuracy across studies. Certain selected studies failed to properly control the confounding variables, which could result in over- or underestimations of the findings.

CONCLUSION

Thrombocytopenia may play a role as a risk factor in the development of retinopathy of prematurity (ROP). Low thrombocyte levels in premature infants have the potential to serve as a clinical biomarker for type 1 ROP detection. However, further research is required to determine the critical platelet count threshold for ROP.

Acknowledgment

We express our gratitude to the team, the reviewers who evaluated our work, as well as to all those who provided support for the conduct of this research.

Conflict of interest

None.

Funding disclosure

None.

Author contribution

IWES contributed to the conception and design. NMAS contributed to the conception and design as well as the analysis and interpretation of the data. PAA contributed to the drafting of the article, critical revision of the article for important intellectual content, and collection and assembly of data. PD contributed to the drafting of the article as well as the collection and assembly of data. SA contributed to the drafting of the article as well as the collection and assembly of data.

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Systematic Review

THE IMPACT OF SYNTAX SCORES ON THE LONG-TERM OUTCOMES OF CORONARY ARTERY BYPASS GRAFTING AND PERCUTANEOUS CORONARY INTERVENTION FOR LEFT MAIN CORONARY ARTERY DISEASE

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ABSTRACT

The Synergy between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery (SYNTAX) score was designed to predict the post-procedural risk associated with percutaneous coronary intervention (PCI) or surgical revascularization. This study aimed to evaluate the long-term outcomes of PCI and coronary artery bypass grafting (CABG) by comparing several existing studies. A systematic search was performed using the PubMed (MEDLINE) and ScienceDirect databases. This systematic review included studies that examined differences in the outcomes of PCI and CABG for left main coronary artery (LMCA) stenosis. This was a systematic review study in which we reviewed original cross-sectional and cohort studies. The search was conducted from February 1st until February 2nd, 2023. The quality assessment of the studies was carried out using the criteria outlined in the Newcastle-Ottawa Scale (NOS). According to the final assessment, all the original research included had a mean NOS score of 8, indicating excellent quality. The literature search yielded 1,675 studies, five of which were selected for the final analysis. A total of 5,494 patients underwent PCI and CABG. This study found that there were variations in outcomes among patients with low, medium, and high SYNTAX scores for long-term major adverse cardiac or cerebrovascular events (MACCE) and long-term mortality. However, similar outcomes were observed in long-term revascularization, long-term stroke, and long-term myocardial infarction (MI). This study concluded that patients with LMCA stenosis and SYNTAX scores ranging from low to high may have different long-term outcomes. CABG is associated with a lower incidence of mortality, repeat revascularization, MI, and MACCE compared to PCI. On the other hand, an association exists between PCI and a lower incidence of stroke.

Keywords: Cardiovascular disease; SYNTAX score; percutaneous coronary intervention; coronary artery bypass grafting; left main coronary artery disease

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Article history
●Submitted 10/7/2023 ● Revised 12/9/2023 ● Accepted 7/11/2023 ● Published 10/12/2023

How to cite: Murteza F, Albab CF, Habibah AT, et al (2023). The Impact of SYNTAX Scores on the Long-Term Outcomes of Coronary Artery Bypass Grafting and Percutaneous Coronary Intervention for Left Main Coronary Artery Disease. Folia Medica Indonesiana 59(4), 418-427, https://doi.org/10.20473/fmi.v59i4.46514

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

Highlights:
1. This study performed a comparison analysis of coronary artery bypass grafting (CABG) and percutaneous coronary intervention (PCI) utilizing the SYNTAX scores, which have been overlooked in prior research.
2. The analysis presents valuable evidence demonstrating the superior effectiveness of CABG in comparison to PCI with regard to long-term outcomes, specifically mortality, revascularization, myocardial infarction, and major adverse cardiac or cerebrovascular events.

INTRODUCTION

In recent years, there have been numerous studies investigating the concurrent use of coronary artery bypass grafting (CABG) and percutaneous coronary intervention (PCI) with stenting. The studies concerned the efficacy of procedures for treating dysfunction in the left main coronary artery (LMCA) (Lee et al. 2016, Park & Park 2017). PCI has been acknowledged as a suitable intervention for

particular patients with LMCA disease. It has been found that PCI is safe and effective for individuals who have low to intermediate anatomic complexity when compared to CABG (Head et al. 2018, Stone et al. 2019).

The 10-year follow-up of the Premier of Randomized Comparison of Bypass Surgery versus Angioplasty Using Sirolimus-Eluting Stent in Patients with Left Main Coronary Artery Disease (PRECOMBAT trial) revealed that there were no significant differences in the use of drug-eluting stents and CABG. Some of the intervention outcomes were mortality as well as severe composite outcomes of death, major adverse cardiac or cerebrovascular events (MACCE), myocardial infarction (MI), and stroke (Park et al. 2020). Several conditions and comorbidities significantly influence the outcome of revascularization. Risk factors such as smoking, alcohol consumption, sedentary lifestyle, diabetes mellitus, and hypertension may negatively impact the outcome of revascularization procedures (Mehta et al. 2019).

The Synergy between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery (SYNTAX) is a tool that researchers developed to help assess the anatomical complexity of coronary artery disease. Additionally, the SYNTAX score can be employed to evaluate the severity of multivessel coronary artery disease (CAD) (Li et al. 2017). The most recent guidelines in the United States and Europe recommend assessing the severity of patients with LMCA disease using the SYNTAX score. After determining the SYNTAX score, clinicians have the option to select the ideal revascularization strategy for the patient. Although the SYNTAX score offers benefits, recent randomized clinical trials have revealed its limitations. The utilization of the tool to compare the outcomes of PCI and CABG is challenging. The patients who enrolled in previous trials had less complex anatomical structures with low to intermediate SYNTAX scores (Mäkikallio et al. 2016, Neumann et al. 2019). Consequently, the findings of these trials could not accurately represent the impact of the SYNTAX scores among the patients. Furthermore, a follow-up duration of less than 5 years was inadequate to ascertain the efficacy of revascularization strategies or assess their long-term effects. Hence, our objective was to review the impact of the SYNTAX score on the long-term outcomes of CABG and PCI for LMCA disease through a comparative analysis of several recent studies.

MATERIALS AND METHODS

In this systematic review, we performed an analysis of multiple cross-sectional and cohort studies. This study reviewed scientific articles that reported the outcomes of CABG and PCI in relation to the SYNTAX scores. The subjects were patients who had been diagnosed with LMCA disease and were monitored with a follow-up duration of at least five years. The exclusion criteria encompassed papers published in languages other than English, studies unrelated to the topic of this systematic review, articles with insufficient or unavailable data, studies with a follow-up duration of less than five years, and duplicates. The data were compiled and analyzed using the 2020 Preferred Reporting Items for Systematic Review and Meta Analysis (PRISMA 2020) (Page et al. 2021).

The data were extracted from a total of 5,494 research subjects documented in five studies, which were published in English-language international publications. We conducted a literature search between February 1st and February 2nd, 2023, to obtain the five research papers. The search was performed on multiple search engines, including PubMed (MEDLINE) and ScienceDirect, using specific keywords "SYNTAX Score AND Outcome AND (PCI OR Percutaneous Coronary Intervention) AND (CABG OR Coronary Artery Bypass Grafting OR Coronary Artery Bypass Surgery) AND (Left Main Coronary Artery OR Left Main Coronary Artery Disease)". We focused on searching for studies that examined the impact of the SYNTAX score on the long-term outcomes of CABG and PCI for LMCA disease. The Rayyan software (https://www.rayyan.ai/) was the tool used for managing the reference sources in this study (Ouzzani et al. 2016).

The studies' quality was assessed according to the criteria set forth by the Newcastle-Ottawa Scale (NOS). A study's quality was deemed "good" if the NOS evaluation resulted in scores of 3 or 4 stars for selection, 1 or 2 stars for comparability, or 2 or 3 stars for outcomes. A study was classified as "fair" if it received a rating of 2 stars for selection, 1 or 2 stars for comparability, or 2 or 3 stars for outcomes. A categorization of "poor" was assigned when the score was 0 or 1 star for selection, 0 star for comparability, or 0 or 1 star for outcomes (Sharmin et al. 2017). The systematic review compared studies that had been evaluated through a rigorous screening process, ensuring that the populations involved appropriately represented patients with coronary artery disease worldwide. In addition, the studies were considered eligible if they exhibited good comparative and exposure characteristics, a sufficient follow-up duration, and a relatively low rate of participant dropout. If the final assessment had a mean NOS score of at least 7, the studies were deemed to be of excellent quality.

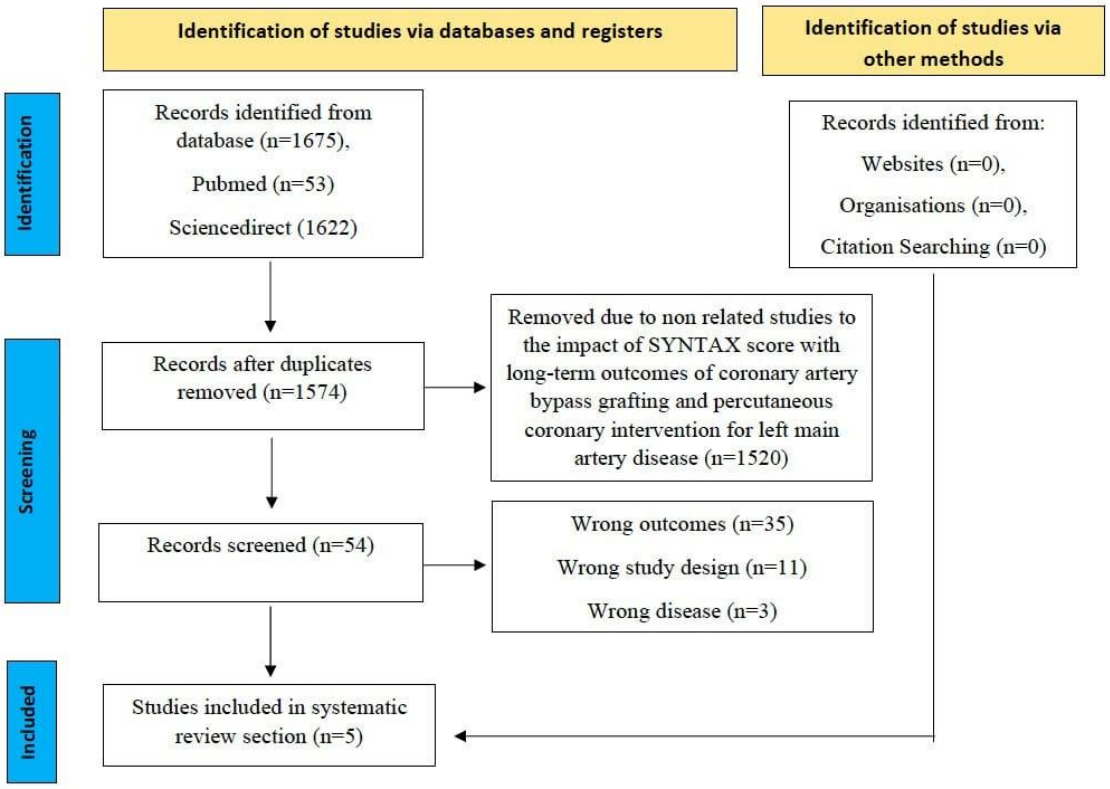

Figure 1. PRISMA flow diagram for the process of article selection.

RESULTS

A total of 1,675 journals were acquired from the electronic databases. After eliminating duplicates and carefully evaluating the titles, keywords, and abstracts, a total of 1,574 papers were identified as not meeting the research's criteria. Furthermore, we excluded 49 journal articles due to inconsistent outcomes, incompatible study designs, and irrelevant diseases. Thus, our analysis only examined five relevant cohort studies (Figure 1).

The five selected studies reported on a total of 5,494 patients, with 2,691 patients receiving PCI and 2,803 patients undergoing CABG. Three of the five papers documented prospective studies, while the remaining two presented a retrospective study and a randomized clinical trial (RCT). The investigations included prospective observational studies, international multicenter randomized trials that utilized the SYNTAX score evaluation, and an open-label RCT known as the PRECOMBAT trial. The SYNTAX score was assessed preoperatively. It was found to be associated with the long-term and late outcomes of interventions for LMCA disease, including mortality, revascularization, MI, stroke, and MACCE. The SYNTAX score was classified into three distinct groups: low, intermediate, and high. Morice et al. (2014) and Yoon et al. (2020) revealed that the incidence of MACCE and its components were identical in patients who underwent PCI or CABG and had low or intermediate SYNTAX scores. Therefore, they merged these groups into a single category.

Table 1 presents a concise summary of the baseline features of the selected studies. It provides a comprehensive overview of the key attributes of the five studies included in this review. The publication dates of all the papers range from 2013 to 2020. A study took place in seventeen countries; another study was conducted in the USA and Europe; two studies were conducted in Korea; and one study was carried out in Japan. A total of 2,691 patients underwent PCI, while 2,803 patients received CABG. Each of these patients was measured for their preoperative SYNTAX score. The follow-up periods varied, ranging from five to ten years. Three studies had a follow-up duration of five years, while two studies had a follow-up duration of ten years.

One study did not mention any details regarding the participants' risk factors. The four remaining studies predominantly consisted of male participants, with an average age of 60 years. The mean age was 59.8±16 for the youngest participants and 69.4±12.1

for the oldest ones. Diabetes, hypertension, and dyslipidemia were among the comorbidities reported by the participants. In three studies, hypertension appeared as the most common comorbidity, with prevalence rates of 50.44%, 51.7%, and 85%. Lee et al. (2021) documented the highest prevalence of dyslipidemia at 41.5%, while Yoon et al. (2020) and Shiomi et al. (2015) recorded rates of 34.24% and 22.8%, respectively. The study by Shiomi et al. (2015) showed the highest prevalence of diabetes mellitus at 44.3%, whereas the study by Mohr et al. (2013) captured the lowest prevalence at 26.96%. Another identified risk factor was current smoking, with prevalence rates of 37.61%, 28.9%, and 23.4% reported in three studies.

Table 1. Baseline features of the five included studies.

Authors, publication year	Countries	Populations (n)		Age (years)	Males (%)	HT (%)	Ds (%)	Cs (%)	DM (%)	Follow-up durations
		PCI	CABG							
Morice et al. (2014)	17 countries (MCS)	346	322	NA	NA	NA	NA	NA	NA	5 years
Yoon et al. (2020)	Korea	819	761	60.3±16.3	71.51	50.44	34.24	37.61	32.27	10 years
Lee et al. (2021)	Korea	291	275	59.8±16	76.5	51.7	41.5	28.9	NA	10 years
Shiomi et al. (2015)	Japan	364	640	69.4±12.1	74.5	85	22.8	23.4	44.3	5 years
Mohr et al. (2013)	USA and Europe	871	805	65.1	83.41	NA	NA	NA	26.96	5 years

Notes: MCS=multicenter study; PCI=percutaneous coronary intervention; CABG=coronary artery bypass grafting; HT=hypertension; Ds=dyslipidemia; Cs=current smoking; DM=diabetes mellitus; NA=not available.

Table 2. SYNTAX scores and the long-term outcomes of CABG and PCI for LMCA disease.

Authors, publication year	Study designs	Revascularization methods	SYNTAX scores	Long-term mortality HR (95% CI)	Long-term revascularization HR (95% CI)	Long-term MI HR (95% CI)	Long-term stroke HR (95% CI)	Late MACCE HR (95% CI)
Morice et al. (2014)	International multicenter prospective randomized trial with the SYNTAX trial	PCI (Drug-eluting stents)	Low to intermediate (≤32)	0.71 (0.44–1.14)	1.23 (0.79–1.91)	1.58 (0.63–3.95)	0.35 (0.09–1.35)	0.94 (0.67–1.33)
			High (≥33)	1.23 (0.76–1.98)	3.30 (1.86–5.88)*	1.88 (0.82–4.30)	0.32 (0.07–1.54)	1.78 (1.21–2.63)*
		CABG	Low to intermediate (≤32)	0.71 (0.44–1.14)	1.23 (0.79–1.91)	1.58 (0.63–3.95)	0.35 (0.09–1.35)	0.94 (0.67–1.33)
			High (≥33)	1.23 (0.76–1.98)	3.30 (1.86-5.88)*	1.88 (0.82–4.30)	0.32 (0.07–1.54)	1.78 (1.21–2.63)*
Yoon et al. (2020)	Prospective observational	PCI (Bare-metal and drug-eluting stents)	Low to intermediate (≤32)	1.10 (0.78–1.54)	4.78 (2.59–8.85)*	NA	NA	1.17 (0.84–1.62)
			High (≥33)	1.39 (1.00–1.92)*	8.29 (4.79–14.34)*	NA	NA	1.27 (0.94–1.74)
		CABG	Low to intermediate (≤32)	1.10 (0.78–1.54)	4.78 (2.59–8.85)*	NA	NA	1.17 (0.84–1.62)
			High (≥33)	1.39 (1.00–1.92)*	8.29 (4.79-14.34)*	NA	NA	1.27 (0.94–1.74)
	Multicenter	PCI (Drug-	Low (<23)	1.18	n (%) = 21	n (%) =	n (%)	0.92

Lee et al. (2021)	prospective open-label RCT with PRECOMBAT trial	eluting stents)		(0.64–2.18)	patients (17.4%)	4 patients (3.3%)	= 2 patients (1.6%)	(0.54–1.55)
			Intermediate (23-32)	0.70 (0.36–1.36)	n (%) = 20 patients (21.4%)	n (%) = 2 patients (2.0%)	n (%) = 2 patients (2.3%)	0.70 (0.41–1.20)
			High (≥33)	1.26 (0.65–2.45)	n (%) = 18 patients (33.4%)*	n (%) = 2 patients (3.6%)	n (%) = 1 patients (2.1%)	0.66 (0.37–1.15)
		CABG	Low (<23)	1.18 (0.64–2.18)	n (%) = 10 patients (10.1%)	n (%) = 3 patients (2.9%)	n (%) = 0 patients (0.0%)	0.92 (0.54–1.55)
			Intermediate (23-32)	0.70 (0.36–1.36)	n (%) = 12 patients (13.2%)	n (%) = 1 patient (1.1%)	n (%) = 3 patients (3.3%)	0.70 (0.41–1.20)
			High (≥33)	1.26 (0.65–2.45)	n (%) = 6 patients (10.1%)*	n (%) = 2 patients (3.2%)	n (%) = 3 patients (5.1%)	0.66 (0.37–1.15)
Shiomi et al. (2015)	Retrospective CREDO-Kyoto PCI/CABG registry cohort-2	PCI (Drug-eluting stents)	Low (<23)	n (%) = 26 patients (22%)	n (%) = 48 patients (42.8%)*	n (%) = 5 patients (4.4%)	n (%) = 12 patients (10.9 %)	NA
			Intermediate (23-32)	n (%) = 35 patients (28.8%) *	n (%) = 41 patients (37.1%)*	n (%) = 7 patients (6.4%)	n (%) = 9 patients (8.5%)	NA
			High (≥33)	n (%) = 26 patients (25.7%)	n (%) = 56 patients (60.0%)*	n (%) = 13 patients (13.7%) *	n (%) = 6 patients (6.5%)	NA
		CABG	Low (<23)	n (%) = 25 patients (17%)	n (%) = 20 patients (14.2%)*	n (%) = 1 patient (0.7%)	n (%) = 13 patients (9.2%)	NA
			Intermediate (23-32)	n (%) = 27 patients (16.5%) *	n (%) = 21 patients (12.8%)*	n (%) = 6 patients (4.0%)	n (%) = 10 patients (6.0%)	NA
			High (≥33)	n (%) = 47 patients (20.4%)	n (%) = 36 patients (16.4%)*	n (%) = 10 patients (4.5%)*	n (%) = 19 patients (8.6%)	NA
Mohr et al. (2013)	Prospective randomized clinical trial with nested registries	PCI (Drug-eluting stents)	Low (<23)	0.88 (0.51–1.51)	1.46 (0.99–2.16)	1.79 (0.87–3.70)	0.43 (0.15–1.26)	1.13 (0.83–1.53)
			Intermediate (23-32)	1.10 (0.70–1.72)	2.03 (1.35–3.06)*	3.11 (1.53–6.31)*	0.55 (0.20–1.53)	1.50 (1.11–2.01)*
			High (≥33)	1.84	2.86 (1.93–	2.57	0.89	1.89

			(1.19–2.83)*	4.25)*	(1.31–5.06)*	(0.37–2.16)	(1.43–2.50)*
	CABG	Low (<23)	0.88 (0.51–1.51)	1.46 (0.99–2.16)	1.79 (0.87–3.70)	0.43 (0.15–1.26)	1.13 (0.83–1.53)
		Intermediate (23-32)	1.10 (0.70–1.72)	2.03 (1.35–3.06)*	3.11 (1.53–6.31)*	0.55 (0.20–1.53)	1.50 (1.11–2.01)*
		High (≥33)	1.84 (1.19–2.83)*	2.86 (1.93–4.25)*	2.57 (1.31–5.06)*	0.89 (0.37–2.16)	1.89 (1.43–2.50)*

Notes: MI=myocardial infarction; MACCE=major adverse cardiac or cerebrovascular events; CABG=coronary artery bypass grafting; PCI=percutaneous coronary intervention; HR=hazard ratio; CI=confidence interval; n=number of samples; NA=not available.

Table 2 presents a summary of the SYNTAX scores in relation to the long-term outcomes of CABG and PCI in patients with LMCA disease. The table includes data for follow-up durations of 5 years and 10 years. Among the selected studies, one paper documented a retrospective RCT. Three of the five studies used a prospective research design. Overall, the selected studies employed different research designs, including prospective observational, international multicenter randomized trials that incorporated the SYNTAX score evaluation, and multicenter open-label RCTs involving the PRECOMBAT trial. The results of this study revealed an association between the preoperative SYNTAX scores and the long-term and late outcomes of PCI and CABG. The measured outcomes included long-term mortality, long-term revascularization, long-term MI, long-term stroke, and late MACCE. The SYNTAX scores were categorized into three groups: low, intermediate, and high. Morice et al. (2014) and Yoon et al. (2020) found that the occurrence of MACCE and its components were identical in patients who underwent PCI or CABG with low or intermediate SYNTAX scores. As a result, they merged the two groupings into a single category.

Table 3. Risk of bias assessment using the Newcastle Ottawa Scale.

Study	Selection			Comparability	Outcomes			Total score
	Representativeness of the exposed cohorts	Selection of the non-exposed cohorts	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Follow-up was long enough for outcomes to occur	Adequacy of follow-up of cohorts	
Morice et al. (2014)	*	*	0	*	*	*	*	*	7
Yoon et al. (2020)	*	*	*	*	*	*	*	*	8
Lee et al. (2021)	*	*	0	*	*	*	*	*	7
Shiomi et al. (2015)	*	*	*	*	*	*	*	*	8
Yoon et al. (2020)	*	*	*	*	*	*	*	*	8

Note: *=the symbol denotes the presence of an assessment component in the study under evaluation.

Table 3 shows the risk of bias assessment for each study. All the studies included in the analysis exhibited a reliable selection process, as the study populations adequately represented the impact of the SYNTAX score on the long-term outcomes of CABG and PCI for LMCA disease. In addition, the studies demonstrated good comparative and exposure aspects. The final evaluation of the studies resulted in a mean NOS score of 8, signifying that the follow-up durations were sufficient and the dropout rates were reasonably low. No bias or issues were found in the measurement or classification of outcomes in any of the studies. The statistical

analysis provided in each study was determined to have excellent methodological quality.

DISCUSSION

The SYNTAX scores in the studies exhibited the capability of assessing the severity and complexity of CAD. The data were organized using a scoring method (He et al. 2020). The European Society of Cardiology/European Association for Cardio-Thoracic Surgery (ESC/EACTS) guidelines on myocardial reperfusion recommend assigning a class I indication for CABG to patients who have LMCA disease and a SYNTAX score ranging from low to high. In addition, the guidelines recommend assigning class IIA and class III indications for PCI to patients who are diagnosed with LMCA disease and have intermediate and high SYNTAX scores (Neumann et al. 2019). Patients with high SYNTAX scores who underwent CABG were found to attain higher survival rates. The general trend seen in the studies indicated comparable findings, as four out of five studies showed lower long-term mortality rates in patients with a high SYNTAX score who underwent CABG compared to those who underwent PCI with a high SYNTAX score. Patients with moderate to high SYNTAX scores and multivessel CAD, but without a diagnosis of LMCA disease, have also exhibited a reduced risk of mortality following a CABG procedure (Chew et al. 2022). Coronary artery bypass grafting (CABG) is the preferred method of revascularization for patients with complex coronary disease. CABG has emerged as the first-line treatment option since the initial SYNTAX trial. PCI is an alternative for CABG in individuals exhibiting a low to intermediate SYNTAX score. Individuals with higher SYNTAX scores exhibited a more favorable outcome following the CABG procedure (Naganuma et al. 2014, Jahangiri et al. 2020).

According to the findings of this systematic review, PCI resulted in more favorable outcomes, particularly in terms of reduced long-term strokes. Among the five studies that examined the comparison between the outcomes of PCI and CABG, three studies found that the PCI groups had lower incidences of stroke, regardless of the SYNTAX score categories. While the results of different research may vary, it is widely accepted that individuals who have undergone CABG are at a higher risk of stroke compared to those who have undergone PCI. Differences in the occurrence rate may be influenced by a reduced risk of stroke in individuals who undergo PCI within the initial 30 days after the procedure (Head et al. 2018b).

Contrary to the outcomes of PCI in terms of long-term strokes, the frequency of long-term revascularization was more prominent in patients treated with PCI who had low, intermediate, or high SYNTAX scores. Furthermore, the incidence of long-term MI was reduced in the groups that received CABG across all of the SYNTAX score categories. The studies demonstrated a significantly reduced occurrence of late MACCE in the groups that underwent CABG compared to the groups that received PCI. This finding is consistent with a separate investigation that observed an increase in MACCE among patients who received PCI and had a higher SYNTAX score compared to those who underwent CABG (Shlofmitz et al. 2019).

In this study, when comparing patients with a high SYNTAX score who received CABG to those who underwent PCI, the frequencies of outcomes such as long-term mortality, long-term revascularization, long-term MI, and late MACCE were found to be lower in the CABG groups. The aforementioned statement is in accordance with the guidelines established by the ESC/EACTS. As per the guidelines, PCI is advised for patients who have LMCA disease and a low to intermediate SYNTAX score. Nevertheless, this approach is not advisable for individuals with elevated SYNTAX scores, which makes CABG a viable alternative for PCI. CABG is the preferred treatment for individuals with multivessel disease and a SYNTAX score of ≥23, while PCI is the optimal treatment for patients with a SYNTAX score between 0 and 22 (Neumann et al. 2019).

In contrast to the results of this study, the findings of the decade-long PRECOMBAT trial revealed that there were no statistically significant differences in outcomes between PCI and CABG among diverse subgroups of patients with stable CAD across different SYNTAX score categories. The absence of discrepancy was identified in the comparison of various outcomes, including MACCE, mortality, stroke, or MI. The Evaluation of XIENCE Everolimus Eluting Stent versus Coronary Artery Bypass Surgery for Effectiveness of Left Main Revascularization (EXCEL) and the Nordic-Baltic-British Left Main Revascularization Trial (NOBLE) yielded comparable results. The contrasting findings could potentially be attributed to the stringent criteria employed for participant selection in the PRECOMBAT trial. The detailed documentation of the results was not possible due to the lack of information regarding the usage of cardiovascular medications throughout the long-term follow-ups. Additionally, it is worth noting that a higher SYNTAX score could have a significant impact on the outcomes. These data may lack universal applicability, hence potentially affecting the long-term outcomes of the PCI and CABG procedures (Lee et al. 2021).

Shiomi et al. (2015) found that patients with unprotected left main coronary artery disease (ULMCAD) who underwent CABG instead of PCI showed more desirable long-term outcomes, particularly those with more complex anatomical conditions. PCI with drug-eluting stents is an effective option for ULMCAD patients who have a low to intermediate SYNTAX score with minimal anatomical complexity. However, despite the effect of anatomical complexity, the clinical outcomes of CABG tend to remain stable. Therefore, CABG continues to be the preferred treatment option for ULMCAD patients with high anatomical complexity or high SYNTAX scores. When determining the revascularization method, it is undoubtedly crucial to take into account the patient's clinical presentation, including factors such as the type and location of the occlusion and the site of the lesion. Furthermore, it is imperative to engage in extensive discussion with a multidisciplinary team (Takahashi et al. 2020). Overall, PCI is an ideal option for those who have less intricate cardiovascular conditions. Conversely, CABG can be beneficial for patients with more complex medical conditions since it may result in a higher survival rate (Zheng et al. 2016).

Strength and limitations

This study represents the first systematic review that examined the association between the SYNTAX score and the long-term outcomes of CABG and PCI for LMCA disease. An advantage of this study is the valuable insight gained from the comparison of PCI and CABG that incorporated analyses of different SYNTAX scores, a topic that has received limited research attention. A limitation of this systematic review is the variability in the PCI methods employed across the selected studies. The differences were evident in the use of sirolimus-eluting stents in one study, everolimus-eluting stents in one study, and both bare-metal stents and drug-induced stents with different time periods in one study. The other studies did not specify the methods that were used in the procedures. Hence, this might affect the accuracy of the findings pertaining to the overall outcomes of the patients. An additional noteworthy constraint that might influence the results of this investigation is the availability of data on the patients' comorbidities. Some papers did not provide sufficient information regarding patient comorbidities that might impact the outcomes of the procedures.

CONCLUSION

Patients with left main coronary artery (LMCA) disease, regardless of their SYNTAX scores ranging from low to high, may experience various long-term outcomes after undergoing either a percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG). However, CABG has several advantages in terms of its outcomes, such as reduced long-term mortality, decreased long-term need for revascularization, lowered risk of long-term myocardial infarction (MI), and decreased occurrence of long-term major adverse cardiac or cerebrovascular events (MACCE). On the other hand, PCI provides a favorable outcome concerning stroke. It is imperative to consider the patient's clinical presentation and engage in thorough discussions with a multidisciplinary team to determine the most viable procedure for treating LMCA disease.

Acknowledgment

The authors would like to thank the reviewers for their insightful feedback and meticulous evaluation of the manuscript.

Conflict of interest

None.

Funding disclosure

None.

Author contribution

All authors contributed to the conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, and final approval of the article. FM, CFA, ATH, and AFM were also responsible for the collection and assembly of the data.

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