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Potential of Binahong Leaf Extract (*Anredera cordifolia*) for Anemia Treatment in Anemic Rat Model

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Abstract

Anemia, characterized by low hemoglobin (Hb) and erythrocyte counts, can be induced by reactive oxygen species (ROS) or known as hemolytic anemia. Binahong (Anredera cordifolia) has been explored for its potential in managing anemia; yet, its efficacy against ROS-induced anemia remains under investigated. This study, conducted from January to May 2023, aimed to evaluate the potential of Binahong leaf extract (BLE) in treating anemia caused by oxidative stress. Anemia was induced by administering NaNO2 to 24 rats (Rattus norvegicus), followed by the administration of BLE at doses of 50,100, and 200 mg/kgBW for seven days. Hemoglobin levels were measured using Sahli method and erythrocytes count were determined using a Hemocytometer at three stages: pre anemia (HA), before-treatment (H0), and after-treatment (H7). Results showed that BLE significantly increased the Hb level by 1.3g/dL (p=0.000), 3.87g/dL (p=0.034), and 4.53g/dL (p=0.016) at 50 mg/kgBW, 100mg/kgBW, and 200 mg/kgBW, respectively, after treatment. Additionally, a dose of 200 mg/kgBW significally increased the erythrocyte count by 3.84 x 106 L/mm3 (p=0.033). These findings suggested that BLE has the potential to improve Hb levels and erythrocyte counts in ROS-induced anemia, indicating a promising natural approach to managing anemia.

Keywords: *Anredera cordifolia*, erythrocyte, hemoglobin, *Rattus novergicus*

Introduction

The World Health Organization (WHO) estimates that anemia afflicts 2 billion individuals worldwide and is predominantly caused by iron deficiency.[1] In Indonesia, the prevalence of anemia is alarmingly high, with approximately 42% of children under the age of 5 years, 40% of pregnant women, and 30–32% of adolescents experiencing anemia.[1,2]

Anemia presents a significant public health challenge, characterized by reduced hemoglobin, hematocrit levels, and/or erythrocyte count.[3] Changes in these parameters can induce hypoxia and increased oxidative stress, marked by elevated levels of reactive oxygen species (ROS).[3] Iron deficiency has been traditionally linked to anemia cases, but it is essential to acknowledge that oxidative stress can also contribute to its development. Erythrocytes are particularly vulnerable to oxidative stress due to their primary role in oxygen transportation.[4] Within the bloodstream, erythrocytes are constantly exposed to ROS, such as hydrogen peroxide and superoxide, leading to hemolytic anemia.[5] The ROS can oxidize hemoglobin, produce methemoglobin, and cause cell damage through lipid peroxidation, ultimately leading to hemolysis and reduced hemoglobin levels.[5] Currently, anemia is primarily treated with iron-based drugs, which may cause nausea, vomiting, diarrhea, and constipation. Furthermore, the

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primary treatment for hemolytic anemia is blood transfusions, where repeated transfusions can lead to iron accumulation within the body.[5]

The Madeira vine, or *Anredera cordifolia* (*Tenore*) *Steenis*, is a succulent climbing vine member of the Basellaceae family. The Madeira vine, known as Binahong in Indonesia, is a well-known traditional medicinal herb valued for healing wounds and curing various ailments.[6] These plants contain many flavonoids in their leaves, stems, tubers, and flowers, which are potent antioxidants and promote erythropoiesis and immunostimulation.[7] The flavonoids also play a pivotal role in managing anemia by preserving heme, which contains iron ions (Fe^{2+}) crucial for producing hemoglobin.[8] Furthermore, ascorbic acid in Binahong leaves accelerates iron absorption up to four times faster, particularly in acidic conditions.[9,10]

Binahong has long been utilized as a traditional medicine in Indonesia. It has been extensively researched, focusing on its antioxidant and antibacterial characteristics and its use to treat bleeding.[11-13] However, there is insufficient evidence of its efficacy in treating ROS-induced anemia.

Therefore, this research aims to investigate whether Binahong leaf extract can improve anemia, especially oxidative stress anemia, by increasing Hb and erythrocyte counts in anemic models. These results could guide further research into developing Binahong leaf extract as a new treatment for anemia.

Methods

The study received approval from the Medical and Health Research Ethics Committee of the Faculty of Medicine, Universitas Kristen Krida Wacana (UKRIDA), with the approval numbers 1308/SLKE-IM/UKKW/FKIK/KE/VIII/2022 and 1385/SLKE-IM/UKKW/FKIK/KE/X/2022. The experiment used male rats (*Rattus norvegicus*) sourced from IPB. They were in verified good health and consistent morphology, weighing between two and three months. The experimental study was conducted from January to June 2023 in the UKRIDA experimental animal laboratory.

After a seven-day adaptation period, anemia was induced in the rats by oral administration of $NaNO_2$ at 25 mg/200g BW per day for 14 days using a gastric probe.[14] Following the adaptation period, the rats were divided into four groups: a negative control group (K1), a treatment group (P1), a treatment group (P2), and a treatment group (P3). All groups received standard feed daily. The negative control group received distilled water, and the treatment group received ethanol extract of Binahong leaves with 50 mg/kgBW (P1), 100 mg/kgBW (P2), and 200 mg/kgBW (P3). The sample size was determined using the Federer formula: (t-1) (n-1) ≥15, where t is the number of treatment group, and n is the number of samples in each treatment groups. A total of 24 rats were used in this study. Furthermore, blood (1–2 mL) was collected from the rat's retro-orbital sinus using a sterile capillary pipette. The collected blood was then transferred to an EDTA-containing microtube to facilitate subsequent quantification of hemoglobin levels and red blood cell counts.

Binahong extract was prepared by cleaning and air-drying the leaves over five days. Subsequently, the dried leaves were finely powdered using a blender. In a maceration jar, 40 grams of powdered Binahong leaves underwent extraction over two 24-hour intervals using 1000 milliliters of 70% ethanol. The resultant mixture was then filtered using filter paper. The filtrate was evaporated using a rotary evaporator at 220 mmHg pressure and 60°C. Before its use in the experiment, the extract was stored in a sealed, clean container in a refrigerator at 4°C.[15]

The chemical reagents used in this study included 0.1 N hydrochloric acid (HCl) for organic compound hydrolysis, glacial acetic acid (CH_3COOH) for bacterial growth inhibition, sulfuric acid (H_2SO_4) for organic compound oxidation, Mayer's reagent for alkaloid detection, Hayem's solution for hemoglobin level assessment, and ethanol as a solvent.[16,17]

Hemoglobin (Hb) concentration was determined using the Sahli method. Briefly, 2 mL of 0.1 N HCl solution is added to a Sahli tube up to the two-mark. Twenty microliters (µL) of blood are then aspirated into a Sahli pipette up to the 20 µL mark and transferred to the tube containing the HCl solution. Following a 5–10-minute incubation for hematin formation, distilled water is added dropwise until the sample color matches a standardized Hb solution within the Sahli tube. The Hb level was then read from the meniscus of the liquid on the g% scale of the Sahli tube, representing the Hb concentration in grams per 100 mL of blood.[18]

Erythrocyte counts were determined using an improved Neubauer hemocytometer and a red blood cell pipette. Blood was drawn to the 0.5 mark in the pipette, diluted with Hayem's solution to the 101 marks, and mixed vigorously for 3–5 minutes. After discarding the first

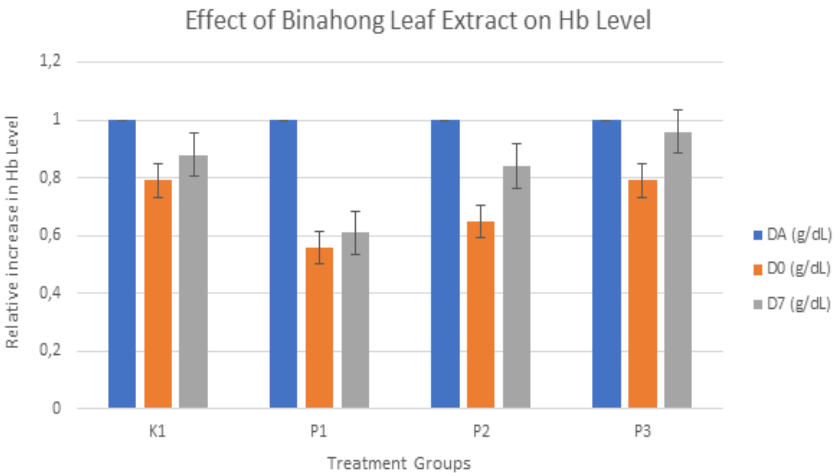

Figure 1 Relative Increase in Hemoglobin (Hb) Levels Following Binahong Leaf Extract (BLE) Treatment

Groups: negative control (K1, distilled water), and treatment groups receiving BLE at 50 mg/kgBW (P1), 100 mg/kgBW (P2), and 200 mg/kgBW (P3)

two drops, a blood droplet was placed on the hemocytometer and covered with a coverslip. Erythrocytes were counted under a microscope at 400x magnification within five large squares, and the total count was calculated considering the dilution factor and volume of the large squares.[18]

Statistical analysis was conducted to examine the average erythrocyte count and Hb levels at different time points before the sodium nitrite administration (DA), 14 days after the administration (D0), and seven days after the treatment (D7). The data was initially assessed for normality and homogeneity using the Shapiro-Wilk test. A normal distribution was considered if the p-value was >0.05, and homogeneity was considered if the p-value was >0.05. A paired t-test was used to evaluate the dependent data. If the p-value was <0.05, H1 was accepted, and H0 was rejected, indicating a significant difference between the analyzed data groups.[19,20]

Results

The ethanol extraction process from 40 grams of Binahong leaf powder yielded 150 milliliters of extract. To determine the yield, 1 milliliter of extract was oven-dried, resulting in a residue weight of 0.03 grams. This corresponds to an extract yield of 11.25%. Figures 1 and 2 present the mean hemoglobin (Hb) levels and red blood cell (erythrocyte) counts across the four groups at three time points: before sodium nitrite administration (DA), after 14 days of administration (D0), and after seven days of treatment (D7).

The mean differences in hemoglobin (Hb) and red blood cell (erythrocyte) counts for each group are presented in Tables 1 and 2, comparing (1) DA to D0, (2) D0 to D7, and (3) DA to D7.

Table 1 shows the difference in mean Hb levels between the D0 and D7 groups, with the highest value observed in the P3 anemic rat

Table 1 Data of the Difference Mean Hb Level between HA, H0, and H14*

Treatment Groups	ΔHA(g/dL)	ΔH0 (g/dL)	ΔH14
K1	3.00	1.20	1.80
P1	4.80	1.35	3.45
P2	3.87	3.87	0.00
P3	2.60	4.53	-1.93

*: A negative control group (K1) and a treatment group received an ethanol extract of Binahong leaves with 50 mg/kgBW (P1), 100 mg/kgBW (P2), and 200 mg/kgBW (P3)

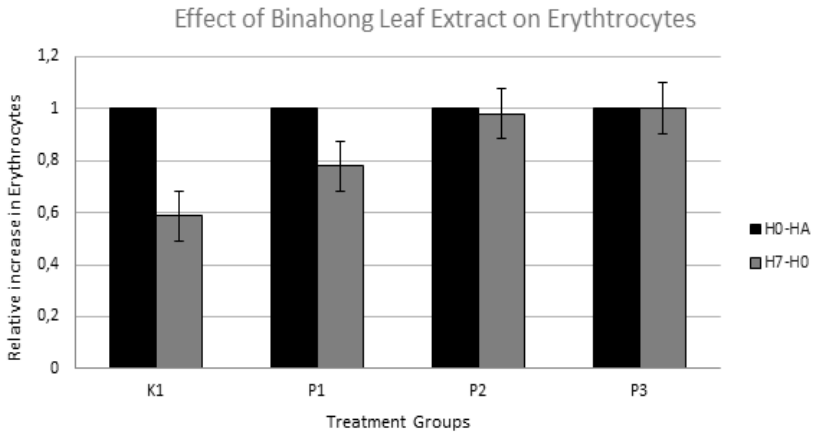

Figure 2 The relative increase in erythrocyte counts after BLE administration compared to the decrease in erythrocyte count in anemia. A negative control group treated using distilled water (K1), a group treated with ethanol extract of Binahong leaves with 50 mg/kg BW (P1), 100 mg/kg BW (P2), and 200 mg/kg BW (P3)

group at 4.53 g/dL. This indicates the capacity of BLE to recover Hb levels to their pre-anemic state and surpass the Hb level of the control group, suggesting optimal improvement of anemia conditions.

Table 2 shows the mean red blood cell count difference between the H0 and H7 groups, with the highest value observed in the P3 anemic rat group at 3.84 x 10^6 L/mm^3 of blood.

The Shapiro-Wilk statistic was used to test the normality of the mean Hb levels and red blood cell count data due to the available data being less than 30. The statistical test revealed that mean Hb levels and red blood cell counts at DA, D0, and D7 were normally distributed, with p-values greater than 0.05. Furthermore, according to Levene's test statistic, the data showed homogeneous variance for all groups (DA, D0, and D7) with a p-value greater than 0.05.

A paired t-test was used to see if there were any differences in the mean Hb and erythrocyte counts for each group between D0 and D7. The paired t-test results for the mean Hb levels between D0 and D7 in the P1, P2, and P3 groups had a p-value less than 0.05, indicating that the administration of ethanol extracts of Binahong leaves can increase the Hb levels in white rats. However, the paired t-test results for the mean Hb levels between D0 and D7 in the K1 groups had a p-value greater than 0.05, indicating no significant variations in mean Hb levels between D0 and D7, as shown in Table 3.

The paired t-test results for mean erythrocyte counts between D0 and D7 in each group had a p-value greater than 0.05, indicating no significant differences between D0 and D7 for all groups except the P3 group. The paired t-test results of the P3 group had a p-value less than 0.05, indicating a significant difference in mean erythrocyte counts between D0 and D7, as shown in Table 4.

Table 2 Data of the Mean Erythrocyte Count Difference between HA, H0, and H7*

Treatment Groups	ΔHA (µLx10^6)	ΔH0 (µLx10^6)	ΔH14 (µLx10^6)
K1	3.87	2.27	1.60
P1	2.94	2.29	0.65
P2	3.03	2.97	0.06
P3	3.84	3.84	0.000

*: A negative control group (K1) and a treatment group received an ethanol extract of Binahong leaves with 50 mg/kg (P1), 100 mg/kgBW (P2), and 200 mg/kgBW (P3)

Table 3 Statistical Analysis Results of Mean Hb Level*

Effect of Treatment	Mean±SD (g/dL)	p-value
K1		
Day-0	11.40±0.14	0.495
Day-7	12.60±0.85	
P1		
Day-0	14.65±1.59	0.000**
Day-7	16.00±1.58	
P2		
Day-0	13.33±1.90	0.034**
Day-7	17.20±1.22	
P3		
Day-0	12.47±1.30	0.016**
Day-7	17.00±1.22	

*: A negative control group (K1), a treatment group, received ethanol extract of Binahong leaves with 50 mg/kgBW (P1), 100 mg/kgBW (P2), and 200 mg/kgBW (P3). **: SD = standard deviation; p<0.05 with paired t-test

Discussion

Anredera cordifolia leaf powder was extracted using 70% alcohol to extract chemical components such as flavonoids, ascorbic acid (Vitamin C), and other substances.[18] The extraction process produced 150 mL of extract from 40g of *Anredera cordifolia* leaf powder, with a yield of 11.25%, which means the ratio of the final product's dry weight to the raw material's weight. A higher yield indicates a higher concentration of extracted chemicals from the raw material.

Nitrate (NO_3^-) and nitrite (NO_2^-) are inorganics ions naturally present in the nitrogen cycle. Exposure to nitrate or nitrite can occur through various sources, such as well water, food, workplace environment occupational exposure, and certain medications. In this study, the rats were given sodium nitrite ($NaNO_2$) for 14 consecutive days at a dose of 25 mg/200g body weight to induce anemia, characterized by reduced hemoglobin levels and a drop in red blood cell count across all treatment groups. Sodium nitrite is used to produce nitrite ions (NO_2^-) and increases the oxidation of the ferrous iron (Fe^{2+}) in deoxyhemoglobin to the ferric (Fe^{3+}) producing methemoglobin and triggers oxidative stress and cellular damage, including red blood cell damage, which further contributes

Table 4 Statistical Test Results of Mean Erythrocyte Counts Data

Effect of Treatment	Mean ± SD ($\mu L \times 10^6$)	p-value
K1		
Day-0	4.31±0.76	0.095
Day-7	6.58±1.40	
P1		
Day-0	5,05±0.65	0.235
Day-7	7.34±1.57	
P2		
Day-0	5.63±1.23	0.109
Day-7	8.61±1.31	
P3		
Day-0	5.65±1.26	0.033**
Day-7	9.49±1.05	

*: A negative control group (K1), a treatment group, received ethanol extract of Binahong leaves with 50 mg/kg BW (P1), 100 mg/kg BW (P2), and 200 mg/kg BW (P3). **: SD = standard deviation; p<0.05 with paired t-test

to the development of anemia.[14,22] Under normal circumstances, only small amount of iron oxidizes to the ferric (Fe^{3+}) state during the routine delivery of oxygen to tissue and the maintenance of methemoglobin levels is primarily facilitated by cytochrome b5 reductase (CYB5R) and the NADH-dependent methemoglobin reductase system.[23]

The CYB5R enzyme is involved in reducing methemoglobin by catalyzing the reduction of methemoglobin (Fe^{3+}) back to functional hemoglobin (Fe^{2+}), which is important for oxygen transport in red blood cells.[20] However, exposure to nitrite ions, which caused an increase in ROS in this study, decrease CYB5R activity through ROS interaction with the heme-binding domain of CYB5R.[20]

Previous research shows that Binahong leaf extract is rich in antioxidants such as flavonoids, ascorbic acid (Vitamin C), and other compounds.[18] The presence of these large amounts of antioxidants is expected to restore the oxidant-antioxidant balance and prevent oxidative stress, optimizing the function of CYB5R in maintaining blood levels methemoglobin in the body and provides a conducive environment for the formation of healthy red blood cells, which in turn improves the previous anemia condition.

Based on the results of this study, *Anredera cordifolia* leaf extract is appears to be effective in treating anemia in P3 group mice, showing a significant increase in Hb levels and erythrocyte counts, particularly at a dose of 200 mg/kgBW. It is suggested that antioxidants such as flavonoids, ascorbic acids, and other compounds in *Anredera cordifolia* leaf extract are believed to improve anemia conditions caused by ROS.

However, the underlying mechanisms have not been explored, and further research is needed to precisely understand the processes that underlie the improvement of anemia with *Anredera cordifolia* leaf extract administration.

In conclusion, Anredera cordifolia leaf extract, particularly at a dose of 200 mg/kgBW, effectively improves hemoglobin levels and erythrocyte counts in rats with sodium nitrite-induced anemia.

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Maternal Anemia in Patients with Preterm Delivery in Indonesia

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Abstract

Preterm delivery remains one of the leading causes of fetomaternal morbidity and mortality worldwide. Anemia during pregnancy is also a significant concern, and may contribute to adverse pregnancy outcomes including preterm birth, intrauterine growth restriction (IUGR), and postpartum hemorrhage (PPH). This study aimed to assess the prevalence of maternal anemia in women with preterm delivery and to evaluate its association with preterm-related complications. A retrospective cross-sectional study was conducted at the Obstetrics and Gynecology Department of Arifin Achmad Hospital, Pekanbaru, Indonesia. Anemia was defined according to the CDC criteria as hemoglobin levels below 11 g/dL in the first and third trimesters or below 10.5 g/dL in the second trimester. Data were collected from medical records of 654 preterm deliveries, of which 359 met the inclusion criteria. Among these, 204 women (56.82%) had anemia, while 155 women (43.18%) had normal hemoglobin levels. A significant association was found between anemia and preterm delivery (p=0.010). Maternal anemia was associated with preterm premature rupture of membranes (PPROM) (p=0.035, contingency coefficient=0.110, 95% CI). These findings suggest that maternal anemia is significantly correlated with preterm birth and its complications, particularly PPROM. Screening and early management of anemia in pregnancy may help reduce the risk of preterm delivery and improve maternal and neonatal outcomes.

Keywords: Anemia, hemoglobin, preterm birth, pregnancy complications, premature rupture of fetal membranes

Introduction

Preterm delivery remains one of the leading causes of maternal and perinatal morbidity and mortality worldwide.[1] Each year, more than 15 million preterm births occur globally, where 60% of the occurrence occurs in African and Asian countries. In 2015, Indonesia ranked ninth among countriese highest number of preterm births, with a national incidence exceeding 15%[2] The 3rd target of the Sustainable Development Goals (SDG) aimed to ensure healthy lives and promote well-being for all ages. Appropriate action in prevention, management, and care for preterm babies can reduce infant mortality and morbidity, along with reducing economic burden of preterm birth for families and the national health system.

The World Health Organization (WHO) has issued various approaches to reduce preterm delivery, including nutritional support for pregnant women.[2]the amount of protein, calcium, and phosphorus become inadequate for the growth of preterm infants, such that fortification is recommended when enteral feeding is established. Recently, intestinal obstruction due to calcium soap formation has been reported. All the reported cases were fed fortified thawed human milk. It has not been elucidated how human milk fortifier reacts with milk fat globules (MFGs Maternal Anemia is one of the main health problems that can be addressed with nutrition support. Centers for Disease Control and Prevention (CDC) defines anemia as a hemoglobin level below 11 g/dL (hematocrit <33%) in the first and third trimesters, or a hemoglobin concentration below 10.5 g/dL (hematocrit <32%) in the second trimester.[3] WHO estimates that 35-75% of pregnant women in developing and 18% in developed countries suffer from anemia.[3] However, although previously suspected, the relationship between preterm delivery and anemia is still not clearly

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defined.[4,5]

A study by Ren et al. showed a relationship between anemia in the first trimester with risk of preterm delivery, IUGR, and low birth weight.[6,7] This study compares the prevalence of anemia in women with preterm delivery. However, further research is needed to validate this relationship, particularly in the Indonesian population. To support holistic care and evidence-based clinical practice in the local context. this study aimed to compare the prevalence of maternal anemia in women with preterm delivery and explore its potential association with preterm-related complications.

Methods

This was a retrospective cross-sectional observational study conducted at the Obstetrics and Gynecology Department of Arifin Achmad Hospital, Pekanbaru, Indonesia. The study protocol was reviewed and approved by the Ethical Review Board for Medicine & Health Research, Medical Faculty Riau University, Indonesia. Data were collected from medical records of patients who delivered preterm from July 2018 to February 2021. A total of 654 patients aged ≥16 who gave birth prematurely (gestational age <37 weeks) based on the patient register data was enrolled in this research. Exclusion criteria includedmultifetal pregnancies, polyhydramnios, poor obstetric history with a history of cervical incompetence, uterine or other abnormalities, and termination with medical indications for both mother and fetus are excluded.

Inclusion patients were classified into anemic and non-anemic groups based on their hemoglobin levels. The limit value of hemoglobin in this study used CDC which defines anemia as a hemoglobin level below 11 g/dL (hematocrit <33%) in the first and third trimesters, or a hemoglobin concentration below 10.5 g/dL (hematocrit <32%) in the second trimester, considering the hemoconcentration condition in the second trimester of pregnancy.

Statistical analysis was performed using SPSS version 25.0. One sample Chi-Square test was used to evaluate the prevalence of anemia among patients with preterm delivery. Additional analyses assessedgestational age at delivery, the association between maternal anemia and the premature rupture of membranes, and differences in leukocyte counts between groups. A p-value <0.05 was considered statistically significant.

Results

Of the 654 patients registered for preterm delivery, 359 met the inclusion criteria and were included in the data analysis. A total of 204 (56.82%) women had low hemoglobin concentrations, compared to 155 (43.18%) women with normal count. A statistically significant number of premature delivery patients with anemia was found (p=0.010). Patients were grouped into Anemia and Non-

Table 1 Baseline Characteristics of Study Participants

Characteristics	Mean ± SD		Frequency (n)		p-value
	Anemia (n=204)	Non-anemia (n=155)	Anemia (n=204)	Non-anemia (n=155)	
Age	29.4±6.5	28.7±6.3			0.706*
Gestation					
First			60	63	
Second			48	32	0.168†
≥Third			92	58	
No data			4	2	
Education					
Elementary			14	14	
Primary School			40	23	0.054†
High School			94	69	
Bachelor			46	48	
No data			10	1	

*Independent t-test; † Chi-square test

Table 2 Analysis of Anemia in the Study Population

	Frequency (n)		p-value	Contingency Coefficient
	Anemia (n=204)	Non-Anemia (n=155)		
Preterm Classification				
Extremely Preterm	14	24	0.031*	0.138
Very Preterm	57	40		
Late Preterm	133	91		
PPROM				
PPROM	115	70	0.035*	0.110
Non-PPROM	89	85		

* Chi-square test

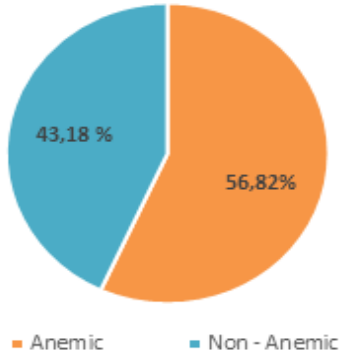

Figure 1 Prevalence of Anemia in Patients With Preterm Delivery

Anemia groups.

Hemoglobin and leukocyte levels were analyzed between the groups. Patients in the anemia group showed significantly lower hemoglobin (Hb) levels, with an average level of 9.55 g/dl (p=0.001). However, there is no difference in the leukocyte count between the two groups (p=0.142).

Baseline characteristics between both groups were analyzed, with parameters such as

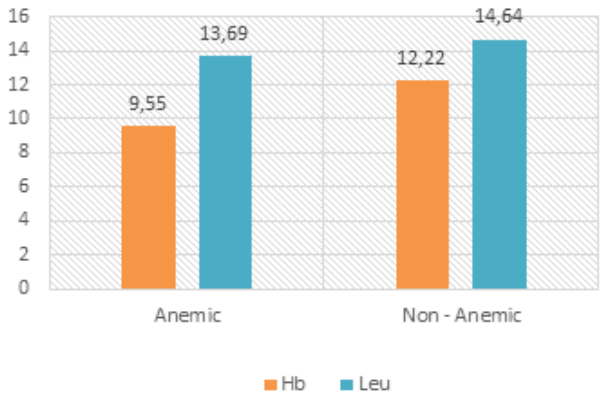

Figure 2 Hemoglobin and Leukocyte Levels in Patients With Preterm Delivery

* p=0.010 for hemoglobin (independent t test);† p=0.142 for leukocytes (independent t test)

age, gestation, and education level. There is no meaningful difference in baseline characteristics between both groups. It can be concluded that age, gestation, and education did not influence the incidence of anemia among premature birth patients.

In this study, most preterm deliveries occurred during the late preterm period, with a statistically significant difference between groups (p=0.031). Furthermore, a significant association was found between anemia and the occurrence of preterm premature rupture of membranes (PPROM) (p=0.035, Contingency Coefficient = 0.110, 95% CI).

Discussion

Preterm labor is a major reproductive health and half of the total cases are with unknown causes.[8] Many studies regarding the relationship between anemia and adverse pregnancy outcomes have ended up with inconsistent findings. Baseline characteristics between both groups were analyzed, with parameters such as age, parity, and educational level. There's no important distinction of pattern characteristics between both groups. It concluded these parameters did not impact the frequency of anemia among preterm birth patients. This finding is different from the findings in other studies that found a strong association between preterm birth, anemia and maternal factors such as sociodemographic, psychological, genetic, paternal, and environmental elements. A study from Khezri[8] reported a significant relationship between preterm delivery and spousal age that might be due to the influence of genetic factors that contribute to the incidence of anemia and preterm delivery. The finding of no correlation between educational status and the anemia event in preterm delivery is also inconsistent with the finding of a previous study that education contributes to mothers' lifestyle and nutritional status related to better knowledge about maternal health. The discrepancies in this finding may be due to the strong heterogeneity in the risk profile of anemia and the incidence of preterm birth. Studies indicate that anemia that contributes to the increased risk of preterm birth is the one that starts from the first trimester of pregnancy and this one is correlated with the knowledge and longer exposure window.[9,10]

This study found that 56.82% of women with preterm labor had anemia. This supports previous findings by Kemppinen et al., who reported that anemia in pregnancy significantly increased the risk of preterm delivery (OR 1.90; 95% CI: 1.15–2.81) and preterm birth itself associated with an increased risk of neonatal mortality and morbidities such as impaired cognition, poor nutrition, neurological disorder and other chronic conditions that may lead to death.[11–13] Khezri et al.[8] also found also found a strong relationship between anemia and preterm birth. Anemia during pregnancy had about 2.69 higher odds of becoming preterm delivery compared to the non-anemic groups.

The timing and duration of anemia appear to be important. Anemia that appeared in all three trimesters of pregnancy was related to an increased risk of preterm delivery. On the other hand, anemia in mid-and late pregnancy was related to diminished ones. There were patterns of increased risk for PPROM in connection to anemia presentation in the early half or all through pregnancy. Anemia in early pregnancy or all through pregnancy may represent pre-existing, or early onset and determined iron deficiency state rather than hemodilution. In turn, it seems to actuate maternal contamination, hypoxia, and oxidative stress, and trigger the unconstrained onset of preterm birth.[10]

Anemia increases the risk of preterm birth by inducing low-grade chronic hypoxia, which reduces nutrition and oxygen-delivery capacity, causing an imbalance in placental oxygen and fetal demand and resulting in fetal stress. In addition, anemia might increase the risk of maternal stress and the release of Corticotropin-Releasing Hormone (CRH). It can cause a series of effects that lead to the activation of labor initiation.[14,15]

This study also found a statistically significant association between anemia and the incidence of PPROM (p=0.035 This is consistent with findings from previous studies, including one in Belitung, where anemic pregnant women were found to have a threefold increased risk of PPROM. Other studies have reported that approximately 59% of anemic pregnant women experienced membrane rupture.[14,15]

Tissue hypoxia, especially in the amniotic membrane will trigger oxidative stress. This situation increases mitochondrial activity and Reactive Oxygen Species (ROS) production. Collagen is the primary target for oxidative stress reactions. Increased ROS will cause the degradation of amniotic membrane collagen and premature rupture of membranes.[14,16]

Within the recent study, a few qualities and restrictions ought to be considered. A solid point

of this study is the sample size which includes 3 years total sample from a referral hospital in Riau province. This study also confined some factors that could interfere with the result of the study. The limitation of this study was this study was conducted retrospectively so there was a limitation in controlling confounding variables. Further research is recommended by performing a multivariable examination thus an obvious relationship between maternal anemia in pregnancy with preterm labor will be clearly defined.

In conclusion, this study demonstrates that maternal anemia is significantly associated with preterm delivery and the occurrence of PPROM. Management plan should address the occurrence, and actively screen and manage co-occurrence of anemia in premature birth patients. PPRoM should be expected as a possible complication of premature birth with anemia.

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HbA1c Level in Type 2 Diabetes Mellitus Patients With and Without Obesity in An Indonesian Regional Hospital

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Abstract

Diabetes Mellitus (DM) is a disease characterized by hyperglycemia due to insulin deficiency or impaired insulin function. The International Diabetes Federation (IDF) estimated that approximately 463 million people aged 20 to 79 years old were affected by DM worldwide in 2019. Indonesia is the third highest with a prevalence of 11.3%. More than 90% of diabetic patients are obese which reduced insulin sensitivity. This study aimed to determine the difference in HbA1c levels between obese and non-obese patients with Type 2 Diabetes Mellitus (T2DM) at Tarakan Regional Hospital, Indonesia. Data from medical records of T2DM patients from January to December 2023 were collected based on inclusion and exclusion criteria using consecutive sampling method, resulting in 128 samples. The results of the study, as determined using the Mann Whitney test, indicated a significant difference in the HbA1c levels between T2DM patients with and without obesity (p=0.031), with non-obese T2DM patients tend to have better HbA1c control compared to their obese counterparts. Thus, it can be inferred that obesity affects the blood glucose control in diabetic patients. However, this result needs to be further researched as there are a few limitations inherent to this study, particularly the partial availability of certain medical record information, such as duration of diagnosis and medication adherenc.

Keywords: HbA1c, Obesity, Type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is a disease characterized by hyperglycemia due to absolute or relative insulin deficiency, impaired insulin function, or both. According to the World Health Organization (WHO), DM is classified into two main types: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM).[1] The difference between these two types is based on age of onset, level of insulin function impairment, level of insulin resistance, and the need for insulin therapy for survival.[2] The International Diabetes Federation (IDF) estimated that in 2019, approximately 463 million adults aged 20–79 worldwide were living with diabetes globally, with a prevalence of 9.3% in that age group. Indonesia ranked seventh worldwide, with an estimated 10.7 million diabetic patients.[3]

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Obesity is defined as an excessive accumulation of body fat, typically occurring when caloric intake exceeds the energy expended through physical activity.[4] In patients with diabetes and obesity, the body has difficulty utilizing insulin produced, a condition known as insulin resistance.[5] Based on Basic Health Research (RISKESDAS) data, it is known that the prevalence of obesity is accompanied by an increase in the prevalence of DM from 2013 to 2018. More than 90% of diabetic patients are overweight or obese. In obesity, there is a decrease in insulin sensitivity, leading to hyperglycemia, which will eventually cause HbA1c levels to increase.[3] Although there is evidence of an association between obesity and increased HbA1c levels in diabetic patients, it is still necessary to measure the specific impact of obesity on HbA1c levels in T2DM patients.

This study addresses whether there is a significant difference in HbA1c values between T2DM patients with and without obesity at Tarakan Regional Hospital and shows the role of HbA1c as an important indicator in controlling long-term glycemic in diabetic patients.[3,6]

Several studies have examined factors that influence HbA1c levels, including the effects of obesity on insulin resistance and subsequent hyperglycemia.[7] However, specific comparative analysis of HbA1c levels between obese and non-obese T2DM patients in clinical conditions is still limited.

This study evaluates the distribution of gender and age among T2DM patients, their body mass index (BMI) profiles, fasting blood glucose (FBG) and HbA1c levels, and specifically the differences in HbA1c levels between obese and non-obese T2DM patients. These objectives aim to improve the understanding of how obesity impacts glycemic control, with the potential to inform clinical practice and increase patient awareness in diabetes management.

Methods

This research used an analytical observational cross-sectional design to compare HbA1c levels based on obesity status in T2DM patients. The research was conducted at Tarakan Regional Hospital in Jakarta throughout 2023. Participants were selected using a consecutive sampling method. A minimum sample size of 64 participants per group (obese and non-obese) was determined using a comparative analytical formula for categorical variables.. Inclusion criteria included a T2DM diagnosis for at least one-year, regular outpatient care, age ≥19 years, and more than one HbA1c test. Exclusion criteria included incomplete medical records, patients under 19, and conditions that could affect HbA1c test results. Data were obtained from patients' medical records, including HbA1c test results (performed according to the hospital's standard laboratory procedures) and anthropometric measurements (height and weight) used to calculate Body Mass Index (BMI). Obesity status was determined using the Asia-Pacific BMI criteria, in which non-obese was defined as BMI <25 kg/m^2 (including pre-obesity with a BMI of 23–24.9 kg/m^2) and obese was defined as BMI ≥25 kg/m^2.[15] This categorization was used to divide participants into obese and non-obese groups. Age was categorized into younger adults (<60 years) and elderly (≥60 years) if required. HbA1c was further classified as well-controlled (<6,5%), moderate (6,5–8%), or poorly controlled (>8%), in line with standard clinical practice.[14] The median HbA1c levels between obese and non-obese patients were compared using the Mann–Whitney U test. A p-value <0.05 was considered statistically significant. All data analyses were performed using statistical software. The study was approved by the Ethics Committee of the Faculty of Medicine and Health Sciences, Krida Wacana Christian University (Approval No. 1669/SLKE/IM/UKKW/FKIK/KEPK/X/2023).

Results

This study was conducted at Tarakan Regional Hospital in Jakarta using secondary data obtained from medical records of T2DM patients from January to December 2023. The research findings are presented as follows. Table 1 shows the distribution of patient characteristics based on gender. The majority of DM patients were female, totaling 68 individuals (53.1%). By age group, there was an equal number of younger

Table 1 Distribution of Study Subjects by Gender, Age Group, and Body Mass Index (BMI)

Characteristics	Total	%	FPG (mg/dL) Median (Min–Max)	HbA1c (%) Median (Min–Max)
Gender				
Male	60	46.9	123.50 (55–279)	6.90 (4.60–15.20)
Female	68	53.1	153.50 (68–336)	8.15 (5.40–12.10)
Age				
Younger Adult	64	50.0	145.50 (78–336)	7.90 (5.00–15.20)
Elderly	64	50.0	123.50 (55–321)	7.25 (4.60–12.70)
BMI				
Without Obesity	64	50.0	123.50 (55–321)	7.10 (4.60–15.20)
Obesity	64	50.0	147.00 (83–336)	8.20 (5.00–12.00)

Table 2 HbA1c Profile in Patients With Type 2 Diabetes Mellitus by Obesity Status

BMI Category		HbA1c (%)		
		Well-controlled	Moderately controlled	Poorly controlled
Obesity	Frequency	9	22	33
	Median (min-max)	6.10 (5.0–6.4)	7.10 (6.5–8.0)	9.00 (8.1–12.0)
Without Obesity	Frequency	20	22	22
	Median (min-max)	6.00 (4.6–6.4)	7.00 (6.5–8.0)	9.05 (8.1–15.2)

adult and elderly patients. The median value (min–max) of FPG based on gender categories was highest among female patients, at 153.50 (68–336) mg/dL. Based on age categories, the highest FPG value was found among in younger adult patients, at 145.50 (78–336) mg/dL. Based on patient BMI, the highest value was recorded in obese patients, at 147.00 (83–336) mg/dL.

The median value (min - max) of HbA1c based on the highest gender category is found in female patients at 8.15 (5.40–12.10) % in line with FPG value of 153.50 (68-336) mg/dL. Based on age categories, the highest HbA1c value is found in younger adult patients, at 7.90 (5.00–15.20)%, consistent with FPG value of 145.50 (78–336) mg/dL. Based on patient BMI, the highest value was recorded in obese patients, at 8.20 (5.00–12.00)%, in line with FPG value of 147.00 (83–336) mg/dL.

Table 2 presents the HbA1c profiles of T2DM patients by BMI category. Among patients with obesity, most had poorly controlled HbA1c levels (n=33), with a median of 9.00%. Only 9 patients in this group had well-controlled HbA1c levels. Conversely, in the non-obese group, most patients had well-controlled or moderately controlled HbA1c values (n=42).

Table 3 shows the statistical analysis using the Mann–Whitney U test. The median HbA1c value in patients with obesity was 8.20% (range: 5.00–12.00%), which was higher than the median in non-obese patients at 7.10% (range: 4.60–15.20%). This difference was statistically significant (p=0.031).

Table 3 Comparison of HbA1c Values Between Obese and Non-Obese Patients With Type 2 Diabetes Mellitus

BMI Category	HbA1c (%) Median (Min–Max)	p-value
Obesity	8.20 (5.00–12.00)	0.031
Without Obesity	7.10 (4.60–15.20)	

Discussion

Based on Table 1, female patients predominated in this study and also had the highest median FPG and HbA1c values.. This finding aligns with a study by Omega et al., which showed a higher prevalence of female DM patients at Mount Maria Hospital in Tomohon.[9] This is supported by the research conducted by Wuni et al., observed a higher frequency of T2DM among female patients in both obese and non-obese groups at Raden Mattaher Hospital in Jambi.[10] This may be related to the higher risk factors for T2DM in females, as women have a greater potential for increased BMI compared to men. Hormonal changes during menstruation, pregnancy, and menopause can also affect glucose metabolism and insulin sensitivity.[9]

Furthermore, the highest median FPG and HbA1c values were observed in the younger adult group. This is consistent with research by Salsabila and Donna, who highlighted that glucose intolerance typically begins around age 45 due to increasing fat content in muscle tissue, which contributes to insulin resistance. When insulin resistance is accompanied by dysfunction of pancreatic beta cells (cells that release insulin), it leads to failure to control blood glucose levels, resulting in an increase in HbA1c levels.[12]

As shown in Table 2, a larger proportion of obese T2DM patients had poorly controlled HbA1c levels compared to non-obese patients. This observation is supported by findings from Wuni et al., which showed that obese patients with uncontrolled HbA1c accounted for 43 (86.0%) individuals, while obese patients with controlled HbA1c accounted for only 7 (14.0%) individuals.[10] This is also supported by a study

by Bernadette and Mohammad, which showed a relationship between BMI and HbA1c values, where high BMI corresponds to high HbA1c values. Obese individuals have a 7-fold higher risk of developing diabetes compared to non-obese individuals. This is because fat cells in obese individuals release pro-inflammatory chemicals that make the body less sensitive to insulin by disrupting the function of insulin-responsive cells and their ability to respond to insulin. Obesity can also trigger metabolic changes in the body, causing adipose tissue to release a number of free fatty acids, proinflammatory cytokines, and other factors involved in the development of insulin resistance. Insulin resistance is accompanied by dysfunction of pancreatic beta cells, it leads to failure to control blood glucose levels, resulting in an increase in HbA1c levels.[11] Obesity alters body metabolism where release of fat molecules from adipose tissue enters the bloodstream, reducing insulin sensitivity of insulin-responsive cells. Fasting blood glucose and HbA1c levels can increase due to lipid accumulation caused insulin resistance.[13] The pancreas continues to produce insulin in sufficient quantities to maintain normal blood glucose levels, but the insulin cannot work optimally to transport glucose into cells due to high cholesterol and triglyceride levels in obese individuals, thus T2DM is closely related to obesity.[10]

Table 3 shows a statistically significant difference in HbA1c levels between obese and non-obese T2DM patients (p=0.031), indicating that obesity is associated with poorer glycemic control.This result is supported by Eka et al.[8] who reported a significant difference (p=0.000) in mean HbA1c levels between obese and non-obese adolescents, with higher values in the obese group. Similar findings were reported by Bernadette and Mohammad, which showed a test analysis result between BMI and HbA1c values with a result of p=0.00.[11] Obese individuals have a strong association with insulin resistance and experience increased cytokines, proinflammatory markers, and other substances involved in the pathophysiology of insulin resistance.[8,11] Obesity alters the body's metabolism, where fatty molecules are released from adipose tissue into the bloodstream, thereby reducing the insulin sensitivity of insulin-responsive cells.[13] The pancreas continues to produce sufficient insulin to maintain normal blood glucose levels, but this insulin cannot function optimally in transporting glucose into cells due to high cholesterol and triglyceride levels in individuals with obesity, which is why Type 2 Diabetes Mellitus (T2DM) is closely linked to obesity.[10] Obesity also impacts pancreatic β cell dysfunction leads to impaired glucose control. Dysfunction of pancreatic β cell leads to inadequate insulin secretion, resulting in higher glucose concentrations in circulation. Continuously increasing glucose concentrations above physiological ranges result in manifestations of hyperglycemia and an increase in HbA1c values.[8,11]

In conclusion, this study demonstrates a significant difference in HbA1c levels between obese and non-obese T2DM patients. It can be inferred that obesity affects the blood glucose control in diabetic patients. However, this result needs to improve further. There are a few limitations, particularly the partial availability of certain medical records such as duration of diagnosis and medication adherence.

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Prevalence, Severity, and Self-Medication for Dysmenorrhea among Female Adolescents in Indonesia

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Abstract

Dysmenorrhea, commonly referred to as menstrual pain, is a prevalent condition affecting many reproductive-aged women, particularly female adolescents, with varying degrees of intensity. Dysmenorrhea affects 64.5% of female adolescents in Indonesia, with the highest prevalence rates found in West Java (98.8%) and Jakarta (87.5%). Over the years, various therapy modalities have been developed for treating this condition based on its pathophysiology. However, in Indonesia, a country renowned for its rich natural resources and medicinal herbs, there is a prevailing belief in the efficacy of traditional medicine. Female adolescents in Indonesia are often engaged in self-medication practices, combining traditional medicine with nonsteroidal anti-inflammatory drugs (NSAIDs). This study aimed to analyze the prevalence, severity, and self- medication practices for dysmenorrhea among female adolescents in Indonesia. The study was conducted from January to December 2020 using an online survey that included questions about menstrual pain, family history, and the use of traditional medicine and NSAIDs. This study comprised 362 participants recruited through convenience sampling. Results showed that 92.5% of participants experienced dysmenorrhea with varying degrees of pain. A total of 216 (59.9%) female adolescents experienced frequent dysmenorrhea, while 118 (32.6%) reported experiencing this condition every menstrual cycle. Of all participants, 33.1% used traditional medicine as a treatment option, and 16.9% used NSAIDs. According to the self-reported visual analog scale (VAS), the use of natural remedies, such as traditional medicine, was associated with lower pain levels, suggesting their potential benefits in healthcare services for dysmenorrhea.

Keywords: Dysmenorrhea, Indonesia, self-medication, traditional medicine

Introduction

Dysmenorrhea, also known as menstrual pain, is a common gynecological condition that affects approximately 70% of female adolescents worldwide.[1–3] In Indonesia, the prevalence is also high, with 64.5% of female adolescents experiencing dysmenorrhea, particularly in urban areas such as Jakarta (87.5%) and West Java (98.8%).[4–8] This finding did not differ from the prevalence of dysmenorrhea among other countries, ranging from 20–93%.[7] The high prevalence can be attributed to its perception as a normal condition, leading to a delay between the onset of symptoms and proper diagnosis.[9,10] This delay has various adverse effects, including reduced reproductive potential and daily functional life. Therefore, early identification and treatment have the potential to reduce pain, prevent disease progression, reduce organ damage, and preserve fertility.[11,12] The severity of dysmenorrhea can significantly impact daily activities. Many adolescents are unable to attend school or participate in regular routines due to

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menstrual pain. Studies have shown that13-51% of adolescents report school absenteeism at least once, with 5–14% experiencing repeated absences.[13] A study conducted in India discovered that the prevalence of dysmenorrhea was high among adolescents (84.2%), with 34.2% experiencing severe pain with VAS 7–10, 36.6% experiencing moderate pain with VAS 4–7, and 29.2% experiencing mild pain with VAS 2–4.[14] Another study conducted in Jakarta, Indonesia found that the majority of adolescents have moderate pain (65.42%), followed by mild and severe pain (22.92% and 1.67%, respectively).[7] Several studies have also shown that dysmenorrhea imposes a significant health and social burden, particularly during productive periods. The impact of dysmenorrhea on the quality of life is profound, manifesting in compromised physical functioning, diminished general health perception, and reduced vitality.[15]

Various treatment modalities are available to manage dysmenorrhea.[11] Nonsteroidal anti-inflammatory drugs (NSAIDs) and oral contraceptives are often recommended as the first-line drugs for treatment. However, their effectiveness can be increased by complementary therapies, such as local heat or regular exercise. Previous reports have also suggested that traditional therapies, such as the consumption of turmeric and ginger can reduce menstrual pain and be developed as alternatives.[6,7] Several traditional herbs, including cinnamon, mint, fennel, coriander, and chamomile, have also been reported to alleviate dysmenorrhea symptoms.[16–18] The treatment of dysmenorrhea in Indonesia has been reported to significantly differ from other countries, particularly regarding the use of alternative and traditional medicine over pain relief drugs.[6,18] The use of traditional medicine is further supported by various in-vitro and in-vivo studies explaining their proposed mechanism of action.[19]

Despite the widespread use of self-medication—particularly the combination of NSAIDs and traditional herbal treatments—among Indonesian adolescents, data on the prevalence and patterns of such practices remain limited. Therefore, this study aims to analyze the prevalence, severity, and self-medication practices related to dysmenorrhea among female adolescents in Indonesia.

Methods

This study used a survey-based cross-sectional design. The sample size was determined using a sample size calculation with a minimal sample size of 96 participants, and participant recruitment was conducted using convenience sampling.

Sample size calculation:

$$\frac{Z\alpha^2 PQ}{d^2}$$

Where:
Zα = standard normal deviate for a 5% significance level (1.96)
P = proportion of the category studied, then it becomes 50%
Q = 1- P, then it becomes 0.50
d = precision, 10%

Meaning:

$$\frac{[(1.96)^2 \times 0.50 \times 0.50]}{(0.10)^2} = 96.04 = 96$$

Participant recruitment was conducted using convenience sampling. The survey was conducted between January 2020 and December 2020, targeting female high school students in Jakarta. The inclusion criteria were: (1) female adolescents aged 14 to 18 years, and (2) those who had already experienced menstruation Jakarta was selected as the study site due to its diversity in ethnicity and socioeconomic status, which offers a representative sample of Indonesian adolescents. An online questionnaire was created using the Google form platform (collected during the COVID-19 pandemic) to collect information on name, age (including birth date and year), dysmenorrhea characteristics, menstrual characteristics, its impact on daily life, specifically the absent days due to dysmenorrhea, additional symptoms, as well as the usage of NSAIDs and traditional medicines. The level of dysmenorrhea was assessed using a self-reported visual analog scale (VAS).

The collected data were analyzed using SPSS Statistics version 25. Descriptive statistics were applied to variables includingage, body mass index (BMI), and first-degree family history of dysmenorrhea. Subsequently, the results were presented using either mean and standard deviation for normally distributed variables or median and range for abnormally distributed variables. The Chi-square test was implemented to evaluate the influence of dysmenorrhea on absenteeism and the utilization of NSAIDs. ROC curve analysis was also presented using independent variables of NSAIDs and traditional

Table 1 Clinical and Sociodemographic Characteristics of Subjects

Variables	Frequency (n=362)
Age (years)*	17 (14–18)
Body mass index (kg/m^2)	20.82 (14.28–37.8)
Underweight	70 (19.3%)
Normal weight	202 (55.8%)
Overweight/Obese	90 (24.9%)
First-degree family history of dysmenorrhea	
Yes	151 (41.7%)
No	211 (58.3%)
Relationship with family history	
Mother	83 (22.9%)
Sister	52 (14.4%)
Both	16 (4.4%)

* median (min–max)

medicine use and the dependent variable of intensity of pain by VAS. The Youden index was then used to determine the optimal cut-off point for the VAS in evaluating treatment effectiveness for dysmenorrhea.

This study was approved by the Health Research Ethics Committee, with ethical clearance letter number ND-1571/UN2.F1/ETIK/PPM.00.02/2020, and was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki. Informed consent was obtained from all participants prior to their inclusion in the study.

Results

A total of 362 participants met the inclusion criteria and were included in the final analysis. The clinical and sociodemographic variables of these subjects are presented in Table 1.The nutritional status of female adolescents was evaluated using body mass index, which yielded a mean of 20.82. A total of 70 patients were classified as underweight (19.3%), 202 subjects were of normal weight (55.8%), and 90 participants were categorized as overweight or obese (24.9%). A first-degree family history of dysmenorrhea was reported by 151 participants (41.7%), which included 83 cases (22.9%) from their mother, 52 (14.4%) from a sister, and 16 (4.4%) from both mother and sister.

The menstrual characteristics of the participants are shown in Table 2. The median age of the first menarche was 12 years, with

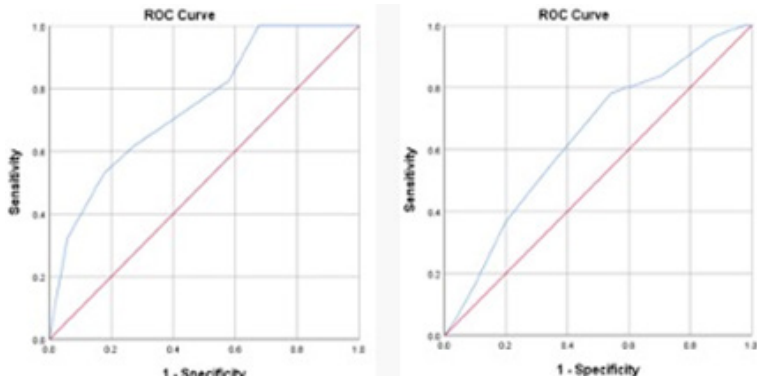

Figure 1 ROC Curve of NSAID Use on Menstrual Pain (Fig. 1a) and ROC Curve of Traditional Medicine Use (Fig. 1b)

Table 2 Menstrual Characteristics of Subjects

Variables	Frequency (n=362)
First menstrual age (years)*	12 (9–16)
Menstrual cycle length	
<24 days	80 (22.1%)
24–32 days	244 (67.4%)
>32 days	38 (10.5%)
Menstrual cycle variation	
≤2 days	89 (24.6%)
3–5 days	187 (51.7%)
>5 days	86 (23.8%)
Menstrual duration	
<3 days	34 (9.4%)
3–5 days	181 (50%)
>5 days	147 (40.6%)
Vaginal discharge	
Yes	314 (86.7%)
No	48 (13.3%)
Regular vaginal discharge	
Yes	138 (43.5%)
No	179 (56.5%)
Menstrual pain	
Yes	335 (92.5%)
No	27 (7.5%)
Menstrual pain VAS*	5 (1–10)
Dysuria	
Yes	33 (9.1%)
No	329 (90.9%)
Dyschezia	
Yes	69 (19.1%)
No	293 (80.9%)

* Median (min–max)

the youngest and oldest being 9 and 16 years, respectively. The results showed that 335 participants (92.5%) had experienced dysmenorrhea with a VAS median of 5, ranging from 1 to 10 among all subjects. However, the majority did not experience dysuria (90.9%) and dyschezia (80.9%).

The impacts of dysmenorrhea on daily life were also recorded, including the usage of NSAIDs and traditional medicine. The results showed that 61 female adolescents (16.9%) had used NSAIDs for dysmenorrhea management. A total of 120 subjects (33.1%) reported the use of traditional medicine, with 116 using turmeric (32.1%), while others used galangal and betel leaf (0.5%). Based on these results, the majority of the subjects used traditional medicine (33.1%) compared to NSAIDs (16.9%) to relieve dysmenorrhea. The impact of dysmenorrhea on daily life was recorded through absence from school due to the condition. A total of 22 participants (6.1%) had recorded absenteeism

Table 3 Impact of Menstrual Pain on Subjects

Variables	Frequency (n=362)
NSAID use*	
Yes	61 (16.9%)
No	301 (83.1%)
Frequency of NSAID use	
Everyday	1 (0.3%)
Once per week	7 (1.9%)
Once per month	11 (3%)
During menstruation	42 (11.6%)
Never	301 (83.1%)
Use of traditional medicine	
Yes	120 (33.1%)
No	242 (66.9%)
Type of traditional medicine	
Turmeric	106 (29.3%)
Galangal	2 (0.6%)
Betel leaf	2 (0.6%)
None	252 (69.6%)
Absent due to menstrual pain*	
Yes	22 (6.1%)
No	340 (93.9%)
Number of absent days	
1	16 (72.8%)
2	3 (13.6%)
3	3 (13.6%)

*Significant variables related to VAS score ($p<0.05$) based on Chi-square test

from school, with 16 reporting only one absent day (n=16;72.8%). The results showed that there was a significant correlation between the use of NSAIDs and absence from school with VAS. The impact of menstrual pain is presented in Table 3.

The receiver operating characteristic (ROC) curve in Figure 1 showed the use of NSAIDs (Figure 1a) and traditional medicine (Figure 1b). The area under the curve (AUC) of each curve was 0.748 and 0.640 for the use of NSAIDs and traditional medicine, respectively. Based on the Youden index, a level of 4.5 was determined to be the specific VAS level needed to use traditional medicine with sensitivity and specificity values of 78.2% and 45.7%, respectively. Meanwhile, a level of 6.5 was determined to be recommended for NSAIDs use with sensitivity and specificity values of 61.8% and 72.5%, respectively.

Discussion

This study includes 362 participants, the majority of whom have a normal body mass index (55.8%). The majority of study participants do not possess an increased risk of having dysmenorrhea, as prior research has demonstrated that obese and underweight females are more prone to this condition.[20] A total of 58.3% of participants do not have first-degree family with menstrual pain. Several conditions increase the risk of developing dysmenorrhea, one of them including positive dysmenorrhea experienced by first-degree family such as mother or sister. The risk of developing dysmenorrhea nearly doubled with a positive family history.[21] This indicates that the majority of our participants are not susceptible to experiencing dysmenorrhea.

Dysmenorrhea poses a significant burden on adolescents' daily lives, affecting physical, emotional, and social well-being..[22] In this study,92.5% of participants report experiencing menstrual pain, with a range of pain severity. Several subjects had additional symptoms of painful urination (9.1%) and defecation (19.1%). These results were consistent with previous studies, which also obtained similar results. A high prevalence rate of dysmenorrhea was reported in West Java by Februanti et al., where a rate of 98.8% was recorded.[8] Another study in France and Stockholm also reported a relatively high prevalence of 92.9% and 89%.[23,24] These results showed that dysmenorrhea was one of the common health problems among female adolescents in the world. However, the variation in the prevalence could be due to the use of different diagnostic tools or perceptions of menstruation.

Based on the results, a total of 33.1% of the subjects consumed traditional medicine, with 32.1% using turmeric. This percentage was higher compared to those who used NSAIDs (over-the-counter painkillers/OTC), with 11.6% taking these painkillers only during menstruation This aligns with the findings of Silaen et al., who report that 75% of female adolescents in Indonesia prefer traditional methods for managing menstrual pain. Both traditional and modern treatments appear effective in alleviating dysmenorrhea symptoms.[6] Dysmenorrhea in this study was reported as a VAS score, and the variable was considered to

have a significant association with the use of NSAIDs. A ROC curve was presented for NSAIDs and traditional medicine usage, and the Youden index was then used. Consequently, it was implied that traditional medicine was commonly used for a VAS of 4.5, while NSAIDs were used for a VAS of 6.5.

A common problem associated with the incidence of dysmenorrhea was decreased concentration and motivation to learn. This often led to an inability to participate in learning activities optimally and absenteeism from school. In this study, 6.1% of the subjects were absent from school due to the condition, with the majority (72.8%) being absent for one day. A similar result was obtained by Silaen et al., where 12.5% of female adolescents were absent from school activities due to dysmenorrhoea.[6]

Consensus guidelines recommend NSAIDs as the first-line treatment for primary dysmenorrhea.[25] The Society of Obstetricians and Gynaecologists of Canada Clinical Practice-Gynaecology and CANPAGO Committees had also published guidelines that recommended NSAIDs use as first-line treatment for spasmodic dysmenorrhoea. This result was also supported by Rafique et al., where 55.8% of the subjects used the drugs to relieve their pain.[26] However, research by Utami et al. [27] showed that some Indonesians were unaware of the effectiveness of NSAIDs.

The limitations of this study are the possibility of bias due to the use of non-probability sampling and the self-reporting nature, which had the potential to cause recall bias and lead to inaccuracies. In addition, the procedures were carried out in several high schools in Jakarta, which might not represent other regions in Indonesia. Further studies using a more generalized population are needed to obtain objective data on the treatment options for dysmenorrhea.

In conclusion, this study identifies a dysmenorrhea prevalence of 92.5% among female adolescents. More participants report using traditional medicine than NSAIDs for pain relief. Based on self-reported VAS scores, traditional medicine is associated with lower pain levels, suggesting it may serve as a beneficial complementary approach in adolescent menstrual health management.

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Effect of Spinal Anesthesia on Blood Pressure During Cesarean Section in Private Indonesian Hospital

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Abstract

The 2018 Indonesian Basic Health Research Data presented an increase in the number of cesarean sections from the previous years, with an increase of 17.6%. In Anugerah Bunda Khatulistiwa Hospital, a private hospital in Pontianak, Indonesia, cesarean section was performed in 689 and 641 cases in 2021 and 2022, respectively. This trend highlights the importance of understanding the anesthesia methods used during this procedure, including spinal anesthesia. Despite the common use of this type of anesthesia, spinal anesthesia an cause changes in the maternal blood pressure. This study aimed to determine the effect of spinal anesthesia on patients' blood pressure during cesarean section. This retrospective observational analytical study was conducted at the Anugerah Bunda Khatulistiwa Hospital Pontianak, Indonesia, from May 2023 to January 2024. Medical records were selected from 246 patients using consecutive sampling method. Most patients aged between 27 and 29 years, with 207 were in the productive age group (20–35 years). The mean weight was 72.77 kg (±10.43) and the mean height was 156.70 cm (±5.85). The majority were overweight based on the BMI score (n=111), and the spinal anesthesia dose used was 15 mg bupivacaine. The Friendman test on the systolic and diastolic blood pressures before and after anesthesia to determine the effect of spinal anesthesia on blood pressure. The results showed a p-value of 0.000 ($p<0.05$) for both systolic and diastolic observations. This indicates that spinal anesthesia influences the blood pressure of patients undergoing cesarean sections under spinal anesthesia.

Keywords: Blood pressure, cesarean section, spinal anesthesia

Introduction

Anesthesia is a loss of the ability to feel pain caused by medication or other medical interventions.[1] There are 3 types of anesthesia: general, regional, and local.[2] In cesarean section, the anesthesia techniques involve either regional or general anesthesia, which has its advantages and disadvantages. The choice depends on the surgery indications, the degree of urgency (urgent circumstances), the mother's conditions, and their wishes.[3] A study conducted at Dr. Kariadi Hospital in Semarang, Indonesia, reported that 85.6% of cesarean sections were performed under regional anesthesia, while only 14.4% used general anesthesia.[4] These figures indicate the increasing trend of regional anesthesia as the primary choice for cesarean sections.[5]

Regional anesthesia, particularly spinal anesthesia, is widely recommended for cesarean sections. This preference is largely due to the risks associated with general anesthesia, such as endotracheal intubation failure and aspiration, as well as its higher potential for maternal morbidity and mortality.[6] As a result, international obstetric anesthesia protocols generally recommend spinal anesthesia for

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cesarean sections in general. This preference is particularly important because fetuses that have experienced asphyxia in the womb will be further affected by the effects of general anesthesia.[7] In developing countries with limited natural resources, spinal anesthesia is the primary choice because it is affordable, relatively safer, and easier to do.[5]

In Indonesia, the rate of cesarean deliveries has increased across both public and private healthcare facilities. Data from the 2013 Basic Health Research (Riskesdas) reported a cesarean section prevalence of 9.8%, which rose to 17.6% by 2018. More recently, a study at Dr. Soedarso Pontianak Hospital in 2021 revealed that there were 527 normal deliveries (53.5%) and 486 cesarean deliveries (46.5%).[8] Similarly, cesarean delivery rates at the Anugerah Bunda Khatulistiwa Hospital in Pontianak continued to increase, reaching 689 and 641 cases in 2021 and 2022, respectively.

One of the risks associated with cesarean sections is a change in the mother's blood pressure. This change is usually a side effect of the use of spinal anesthesia during the cesarean section surgery, both in a sitting and inclined position. Therefore, continuous monitoring for blood pressure, pulse, and oxygen saturation is necessary during this procedure. In spinal anesthesia, rapid changes in blood pressure should be avoided because they can interfere with placental perfusion unless preoperative therapy has been adequately prepared through fluids and vasopressors.[9]

Given the rising prevalence of cesarean sections and the associated risks, this study focuses on the effect of spinal anesthesia on blood pressure. The novelty of this research lies in the extended monitoring periods (pre, 10 minutes, 20 minutes, and 30 minutes), which provide a more comprehensive understanding of blood pressure changes in cesarean section patients..

Methods

To achieve the research objectives, an observational analytical study with a retrospective approach was employed. The study was conducted at Anugerah Bunda Khatulistiwa Hospital, Pontianak, Indonesia, from May 2023 to January 2024. The sampling technique used was consecutive sampling, in which every patient who met the inclusion criteria was included until the required sample size was reached. The sample size was calculated using the Slovin formula, resulting in a total of 246 respondents. Samples were excluded if there were absolute contraindications (patient rejection, infection at the injection site, increased intracranial pressure, severe hypovolemia, and coagulopathy or hemostasis disorders), relative contraindications (sepsis or bacteriosis, uncooperative patients, neurological deficits, spinal deformities, and stenosis heart disease), patients with a history of disease (uncontrolled diabetes mellitus, uncontrolled hypertension, and stroke), (patients epilepsy, arrhythmias, hypotension, and congenital heart disease), patients with incomplete medical record data, twins, and emergency patients.

The study utilized secondary data obtained from the patients' anesthesia records at Anugerah Bunda Khatulistiwa Hospital. Data processing and analysis were conducted using the SPSS software. The Sturges method was applied for data grouping. Univariate analysis was conducted to describe the minimum, maximum, mean, standard deviation, frequency, and percentage of the variables. The variables analyzed included patient characteristics such as anesthetic dose, body weight, height, body mass index (BMI), age, and blood pressure. To assess the effect of spinal anesthesia on blood pressure, a bivariate analysis using the Friedman test was performed. After that, the normality test was employed using the kolmogorov-smirnov test and the results showed that the data was not distributed normally. This research has received approval from the Research Ethics Committee of the Faculty of Medicine, Tanjungpura University, through a Certificate of Passing the Ethical Assessment (Ethical-Clearance) No: 6285/UN22.9/PG/2023.

Results

This section presents the demographic and clinical characteristics of the participants, along with the findings related to spinal anesthesia and blood pressure changes during cesarean section. *T*his study count dose spinal anesthesia with hyperbaric bupivacaine 0.5% respect to patient height The majority of participants (33.33%) had a height of 156–160 cm and received a 15 mg dose. Only 0.41% of patients were height >170 cm with dose 18 mg bupivacaine. Patients height 151–155 cm with dose 14 mg bupivacaine accounted for 29.67%. Patients height 161–165 cm with dose 16 mg bupivacaine made up

Table 1 Dose of Spinal Anesthesia (0.5% Hyperbaric Bupivacaine) According to Patient Height

Patient height (cm)	Dose (mg)	n
140–145	12	9
146–150	13	24
151–155	14	73
156–160	15	82
161–165	16	43
166–170	17	14
>170	18	1

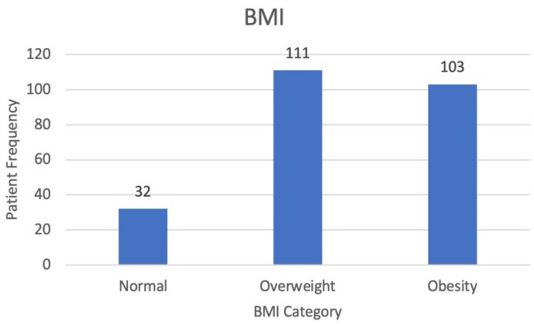

Figure 1 Frequency Distribution of Patients by BMI Category

Table 2 Patient Characteristics Based on Body Weight and Height (n=246)

Variable	n	Minimum	Maximum	Mean	Standard Deviation
Weight (kg)	246	50.50	109	72.77	10.43
Height (cm)	246	140	171	156.70	5.85

17.48%. The height 146-150 cm with dose 13 mg bupivacaine represented 9.76%, followed by 5.69% in the height 166-170 cm with dose 17 mg bupivacaine. Additionaly, 3.66% of the sample was in the height 140-145 cm with dose 12 mg bupivacaine.

Table 2 shows characteristic distribution data on the weight and height of patients in this study. The mean score of the patient's body weight was 72.77 kg. Meanwhile, the mean height of the patients was 156.70 cm.

In addition to these physical characteristics, the research results also provide data on the patients' BMI categories. For example, the largest portion of the sample fell into the overweight category, comprising 45.1% of the participants. This was followed by samples with an obesity category with 41.9%. After that 13.0% of the participants had a normal BMI. There was no sample in the underweight category. Obesity in pregnancy is defined as a BMI of 30 kg/m^2 or more at the first antenatal.

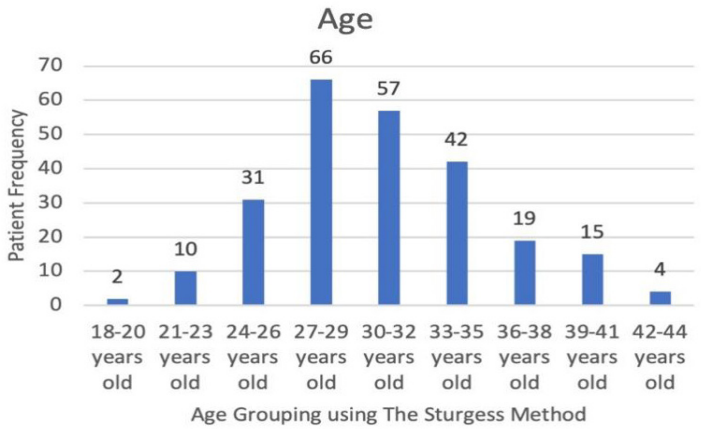

Figure 2 Age Frequency Distribution of Cesarean Section Patients

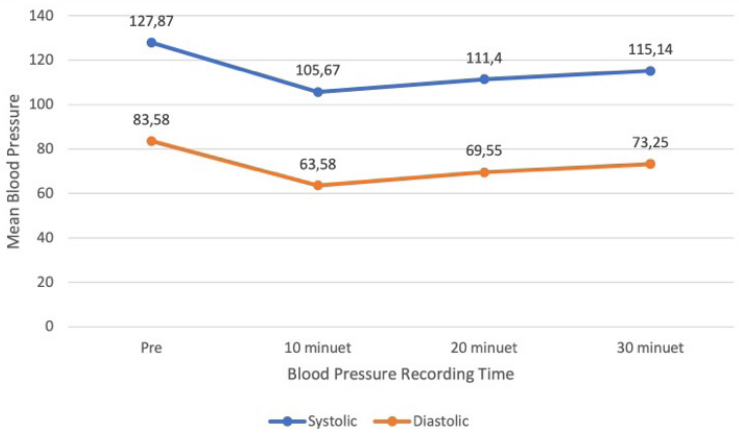

Figure 3 Image of Mean Systolic Blood Pressure And Diastolic Before Administering Spinal Anesthesia, 10th, 20th, And 30th Minutes After the Administration of Spinal Anesthesia

Table 3 Kolmogorov Smirnov Test Result

Variable	Time Points	p-value	Distribution
Systolic	Pre	0.000	Abnormal
	10 minutes	0.000	Abnormal
	20 minutes	0.000	Abnormal
	30 minutes	0.000	Abnormal
Diastolic	Pre	0.000	Abnormal
	10 minutes	0.000	Abnormal
	20 minutes	0.000	Abnormal
	30 minutes	0.000	Abnormal

Furthermore, the study also examined the age distribution of cesarean section patients. Using the Sturgess method, the results showed that most samples were within the 27-29 age range, representing 26.8% of the total sample. Only 0.8% of patients were between 18-20 years old, while 4.1% were in the 21-23 age group. Patients aged 24-26 years made up 12.6%, and those aged 30-32 years accounted for 23.2%. The 33-35 age group represented 17.1%, followed by 7.7% in the 36-38 age range. Additionally, 6.1% of the sample was aged 39-41 years, and 1.6% were between 42-44 years old. The youngest patient was 18 years old, and the oldest was 44 years old. A total of 207 patients, or 84.15% of the samples, were in the productive age range of 20-35 years, while 39 samples (15.85%) fell outside this range.

Following the analysis of patient characteristics, the study also evaluated changes in blood pressure over time. Figure 3 shows the mean blood pressure. There were significant differences in systolic and diastolic blood pressure before and after spinal anesthesia, at the 10th minutes, 20th minutes, and 30th minutes. To assess the distribution of the data, a normality

Table 4 Friedman Test Results for Systolic and Diastolic Blood Pressure

Variable	Time Points	p-value
Systolic	Pre, 10, 20, 30 min	0.000
Diastolic	Pre, 10, 20, 30 min	

test was conducted. The Kolmogorov–Smirnov normality test results for both systolic and diastolic blood pressure variables at all four time points yielded p-values less than 0.05, indicating non-normal data distribution (Table 3). As a result, non-parametric testing was performed.
The Friedman test was used to analyze the changes in blood pressure over the four time points. The results showed a statistically significant difference in both systolic and diastolic pressure after spinal anesthesia, with p-values of 0.000 for each (Table 4). These findings confirm that spinal anesthesia has a measurable and significant effect on maternal blood pressure during cesarean section procedures.

Discussion

Based on Table 1, the administration of spinal anesthesia using 0.5% hyperbaric bupivacaine was adjusted according to patient height. Taller patients required higher doses to achieve the same dermatome level. This is because an increased height typically corresponds to a larger cerebrospinal fluid volume, necessitating a greater anesthetic dose. However, higher doses are associated with a greater risk of hypotension. Bupivacaine works by blocking nerve fibers, including sympathetic nerve fibers which function to regulate vascular tone. As the dose of bupivacaine increases, sympathetic nerve blockade also increases, leading to vasodilation of blood vessels. This vasodilation results in a decrease in systemic vascular resistance and in turn lowers blood pressure. This finding is in line with research carried out by Huang in Shenzen Hospital. The results indicated that the quality of anesthesia and incidence of maternal hypotension are related to block level, which depends on the dose of local anesthetic injected into the subarachnoid space. The volume of the subarachnoid space is decreased in parturients due to high abdominal pressure. When using a low dose of local anesthetic, the block level of the local anesthetic may also depend on the height of the parturient.[10]

The results of the study showed that the average patient weight was 72.77 kg (72.77 kg±10.43). These findings highlight the importance of monitoring maternal weight during pregnancy. Body weight significantly influences the health conditions of both the mother and the baby. Obese women have a higher potential to give birth to obese children, and fetal and neonatal complications are more prevalent in these cases. One such complication is macrosomia., which is defined as a condition in infancy born with a body weight of over 4 kg. Macrosomia is often an indication for a cesarean section. This finding is in line with research carried out by Ekwendi on pregnant women with obesity. The results indicated that higher BMI, weight, and age in pregnant woman are associated with the increased risk of cesarean deliver compared to vaginal.[11]

The second finding of this research was the average height of the patients, which was 156.70 cm (156.70 cm±5.85). This finding was similar to that performed by Alfarisi during a cesarean section at Dr. Mohammad Hosein Palembang in 2020. The research obtained that 282 patients had heights of more than 145 cm, representing 96.9% of the sample.[12] Interestingly, this was different from research conducted by Strong in singleton births in India. This research showed that the shorter maternal height (<145 cm) was associated with a greater risk of cesarean section.[13] Shorter individuals tend to have a narrow pelvis, which can complicate childbirth. Additionally, shorter maternal stature has been associated with pregnancy outcomes, such as babies born with low birth weight and lower APGAR scores (Appearance, Pulse, Grimace, Activity, and Respiration). However, cesarean section indications are not only determined by height as other indications, such as persistent fetal distress, cord prolapse center, and failed vacuum/forceps also play a significant role.[14]

The next result provided by the research was that the largest BMI category was overweight, with 111 people (45.1%) falling into this category. In fact, excessive weight in pregnant women can have various consequences and risks for both the mother and fetus. In overweight pregnant women, obesity can increase the risk of developing gestational diabetes, hypertension, preeclampsia, macrosomia, and weight retention after delivery. It also increases the potential for a cesarean section procedure. The results of this research are supported by those conducted by Liu et al. in 2022. This research showed that pregnant women with an obese BMI had a risk factor for hypotension intraoperative cesarean section. An overweight woman is more susceptible to hypotension due to increased maternal BMI; thus, cerebrospinal fluid gradually decreases. In obese patients, Epidural blood vessels are more enlarged, and a large number of fat deposits cause epidural stenosis. This can cause fetal umbilical artery blood flow to become abnormal, putting the fetus in a state of chronic hypoxia and

ultimately leading to a poor perinatal prognosis. Clinically, pre-infusion fluid expansion is often used to prevent hypotension. At the same time, the supine position is 30 ° left, used to move the uterus to the left, and vasoconstrictor drugs are given to avoid hypotension.[15]

Regarding age groups, this research reported that cesarean section patients were typically between 27 and 29 years, with 66 people (26.8%). The youngest age given this procedure was 18 years old, while the oldest was 44 years old. In addition, most of the samples 84.15% were at a productive age of 20–35 years. This finding confirmed the research by Alfarisi in 2020. The research revealed that 204 (70.1%) mothers who gave birth with cesarean section were within 20–35 years.[12] However, 15.85% of the samples in this study were outside the productive age, with mothers either younger than 20 years or older than 35 years. Both age groups carry additional risks during pregnancy. At a younger age, a woman's reproductive system may not be fully developed, and their psychological growth is immature. On the other hand, women of advanced maternal age are vulnerable to various pregnancy complications, such as diabetes, gestational, and preeclampsia.[12]

The effects of spinal anesthesia on blood pressure during cesarean section procedures are an important aspect to consider in managing patient safety. The study's results of this study showed significant differences in both systolic and diastolic blood pressure before and after the administration of spinal anesthesia at the 10^{th}, 20^{th}, and 30^{th} minutes. These differences occur because of arterial vasodilation, which results in a decrease in systemic resistance. As a result, there is a decrease in cardiac output accompanied by a decrease in heart rate. This finding was in line with Ferre et al. in 2020, which obtained results of hypotension that occurred during anesthesia for cesarean section surgery. This is the most common side effect caused by spinal anesthesia that requires precautions and special treatment. Spinal anesthesia causes hypotension through several pathophysiological mechanisms, with the most significant being the rapid onset of sympatholytic due to the increased sensitivity of nerve fibers to spinal anesthesia.[16]

In line with previous findings on anesthesia effects, the degree of blockage of the sympathetic chain is related to the rate of spinal anesthesia spread to the cranial region, which is often difficult to predict and usually reaches some dermatomes above the level of sensory block. The increased sensitivity to spinal anesthesia, combined with compression of the aortic cavity by the pregnant uterus, is the main reason for the higher incidence and rate of hypotension in pregnant women compared to non-pregnant patients. Furthermore, pregnant women showed increased levels of sympathetic activity compared to parasympathetic activity. Therefore, sympatholysis causes higher levels of peripheral vasodilation and activity dominance parasympathetic, consequently reducing venous return and preload heart. This decrease in preload contributes to a reduction in cardiac output (CO), which causes systemic hypotension.[16]

Understanding the impact of the sympathetic block is crucial as a higher sympathetic block proportionally reduces the occurrence of compensatory mechanisms through baroreceptors and increases the risk of cardioinhibitory reflexes such as Bezold Jarisch Reflex (BJR), which can ultimately cause an attack heart to death. BJR is a cardiovascular and neurological process that causes bradycardia. Additionally, acute hypotension reduces cerebral perfusion, causing ischemia in the temporary brainstem. To mitigate this, oxygen inhalation can help prevent cerebral hypoxia. Furthermore, neonates born to mothers with hypotension due to spinal anesthesia were significantly more acidotic.[16]

To assess the distribution of the data, a normality test was conducted using the kolmogorov-smirnov test. The test revealed that the data was not normally distributed. This deviation is attributed to the presence of a large interval difference.[17] For systolic blood pressure values, the lowest recorded value was 80 mmHg, and the highest value was 150 mmHg at the 30^{th} minute. Similarly, the lowest value for diastolic blood pressure was 40 mmHg, with the highest value at 105 mmHg at the 30^{th} minute.

The findings of this research highlight the significant impact of spinal anesthesia on both systolic and diastolic blood pressure in patients undergoing cesarean section, as indicated by a value of $p<0.05$ from the Friedman test. While spinal anesthesia can be safely administered to cesarean section patients, it may lead to several complications. One of them is a decline in blood pressure. This reduction in blood can affect the health condition of the mother and baby. If this lasts a long time and is unaddressed properly, it can cause tissue hypoxia, decreased consciousness, and even death.[2]

Following the earlier discussion on spinal anesthesia's impact on blood pressure, this study showed significant changes in blood

pressure after the procedure. The results revealed differences in systolic and diastolic blood pressure before and after administering spinal anesthesia, especially at the 10th minute. These changes occur due to the vasodilation of the veins, resulting in the accumulation of blood in the viscera and lower extremities. As a result, there is less resistance in the blood vessels, leading to a decrease in cardiac output and heart rate.[2]

Furthermore, at the 20th minutes, differences in systolic and diastolic blood pressure were noted, indicating that the effect of the sympathetic block from spinal anesthesia is still strong for cesarean section patients during this phase. By the 30th minute, the differences between systolic and diastolic blood pressure began to approach pre-anesthesia (baseline) values. Researchers need to observe up to 30 minutes after anesthesia to ensure patient safety and manage potential hemodynamic complication.

The results of this research were supported by the existing research, for instance, carried out by Elfeil et al. in 2021. The research showed that changing position from a supine to a side position is a significant predictor of blood pressure. However, this is just mildly associated with intraoperative hypotension. Higher sympathetic activity before neuraxial anesthesia is associated with an increased risk of post-spinal hypotension. Meanwhile, large hemodynamic variability after changes in position from a supine to a sideways position indicates sympathetic activity higher in blood vessels. The higher the autonomic activity, the higher the risk of hypotension after spinal anesthesia.[18]

Previous research results also support the current results, as exemplified by Chauhan et al. in 2022. The research revealed that prophylactic use of ephedrine and phenylephrine are both effective in preventing maternal hypotension during cesarean section surgery under soinal anesthesia. Particularly, phenylephrine is superior to ephedrine in treating hypotension, as evidenced by a higher umbilical blood pH value. Ephedrine is a mixed adrenoceptor and agonist. It maintains blood pressure primarily by activating adrenoreceptors and increases cardiac output and heart rate. However, ephedrine can cross the placental barrier and cause an increase in the fetal heart rate and increase fetal catecholamine levels, which may mediate elevated levels of fetal catecholamines. It also mediates increased consumption of oxygen and increased concentrations of glucose and lactic acid. Phenylephrine is a pure adrenergic agonist that can antagonize a decrease in systemic vascular resistance caused by spinal anesthesia.[19]

A limitation of this study is the reliance on handwritten medical records, which slowed the data retrieval and recapitulation process. Despite this, the study concludes that spinal anesthesia influences blood pressure in patients undergoing cesarean sections.

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Prognostic Nutritional Index (PNI) at Admission Predicts In-Hospital Mortality of COVID-19-Infected Patients

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Abstract

Corona Virus Disease-2019 (COVID-19) was declared a pandemic in March 2020 and caused considerable deaths in all parts of the world. Mortality is influenced by the immune system status and increased vulnerability to infection, both related to nutritional status. The Prognostic Nutritional Index (PNI), calculated using lymphocyte count and albumin levels, may have the ability to more accurately characterize the nutritional and inflammatory conditions of COVID-19 patients. This retrospective study analyzed 967 COVID-19 patients at Ulin Hospital Banjarmasin, Indonesia, by examining demographic data, laboratory results, and PNI in relation to survival outcomes. The study revealed that factors such as age, sex, comorbidities (including hypertension, diabetes mellitus/DM, obesity, etc.), number of comorbidities, and disease severity correlated with mortality. Leucocyte count, lymphocyte count, albumin levels, and PNI all showed significant correlations with survival ($p < 0.001$), suggesting that these factors may serve as useful prognostic indicators for COVID-19 patient's survival. The PNI was associated with an increased risk of mortality, with a univariate odds ratio (OR) of 0.923. Receiver operating characteristic (ROC) analysis demonstrated that a PNI cut-off value of <41.9 had a sensitivity of 44.9% and a specificity of 82.4%, with an area under the curve (AUC) of 0.666 ($p < 0.001$). Hence, PNI at admission, which reflects patients' immune system and nutritional status upon hospital admission, can be used as a simple, cost-effective, and reliable predictor of mortality in COVID-19 patients.

Keywords: COVID-19, leucocyte, mortality, prognostic nutritional index, prognosis

Introduction

The World Health Organization (WHO) declared Coronavirus Disease 2019 (COVID-19) a pandemic on March 11, 2020, resulting in extensive global mortality. By the end of 2022, Indonesia's case fatality rate (CFR) was 2.4%, higher than the global CFR of 1%, while South Kalimantan, one of the provinces in Indonesia, had an even higher CFR of 2.9%. COVID-19 severity ranges from mild to critical.[1] The World Health Organization (WHO) declared Coronavirus Disease 2019 (COVID-19) a pandemic on March 11, 2020, resulting in extensive global mortality. By the end of 2022, Indonesia's case fatality rate (CFR) was 2.4%, higher than the global CFR of 1%, while South Kalimantan, one of the provinces in Indonesia, had an even higher CFR of 2.9%. COVID-19 severity ranges from mild to critical.

Previous studies raised concerns about patients with severe pneumonia, who experienced protein loss and impaired immune defenses..[2] Patients with COVID-19 also exhibited protein loss symptoms, including decreased albumin levels and compromised organ function, emphasizing the significance of nutrition risk assessment and its prognostic value for COVID-19 patients. Accumulating evidence suggests that patients infected with COVID-19 have an inferior prognosis if they have significant systemic inflammation and a deficient nutritional status.[3]

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Nutritional screening should ideally be simple and non-invasive. The Prognostic Nutritional Index (PNI), calculated using serum albumin and lymphocyte counts, is a widely used indicator of nutritional, immunological, and inflammatory status. It has demonstrated prognostic value in various conditions, including cardiovascular disease, infections, and cancer.[4,5] Malnutrition weakens the immune system and increases infection vulnerability. Hypoalbuminemia mostly reflects nutritional status, but it has also been linked to the severity of COVID-19. The declining albumin level could indicate a severe cytokine storm and organ damage. As evidenced by albumin levels, the poor nutritional state of COVID-19 patients is unfavorable to tissue repair and recovery. Lymphocyte count, a critical component of PNI, was found much lower in non-survivors. Nevertheless, lymphopenia has been confirmed as an independent risk factor for mortality among COVID-19 patients. The lymphocyte drop could be linked to diminished immunological activity and a substantial cytokine increase. The PNI could more precisely characterize COVID-19 patients' nutritional and inflammatory conditions.[6] Low PNI may suggest severe conditions and a poor prognosis for patients. Therefore, this study aims to determine whether the nutritional status at hospital admission, as assessed by PNI, can predict in-hospital mortality among COVID-19 patients, particularly in resource-limited settings like Indonesia.

Methods

This study used a retrospective design based on medical records of COVID-19 patients treated at Ulin Regional Hospital, the primary referral hospital for COVID-19 in South Kalimantan, Indonesia, between March 2020 and December 2021.

Data were taken using purposive sampling with the inclusion criteria of all medical record data for COVID-19 patients aged 18 years who were treated at Ulin Regional Hospital, Banjarmasin with complete research variables in admission. Variables based on clinical and laboratory data with all adult patients had either hospital discharge or mortality as their definitive clinical outcome. Demographic factors include age, gender, co-morbidities, number of co-morbidities, disease severity, and body mass index (BMI). The patients' blood test parameters, such as lymphocyte and albumin on the first day of admission, were compared to survival. The Prognostic Nutritional Index (PNI) was calculated on the first day of admission using the following formula:

[10 x serum albumin (g/dL)] + [0.005 x lymphocyte count (per mm^3)] 11, on the first day of hospitalization.

A total of 976 patients met the inclusion criteria and were included in the final analysis. Patients were divided into two groups: survivors (those discharged from the hospital) and non-survivors (those who died during hospitalization).

Statistical analysis was performed using SPSS software version 26.1. The Kolmogorov-Smirnov test was used to assess the normality of data distribution. The Mann-Whitney U test was used to compare continuous variables between groups. The Chi-square test was applied for examining independent qualitative data, while Fisher's exact test was utilized when the conditions for the Chi-square test were not met. Univariate logistic regression was performed to estimate the risk factors for mortality. T Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic value and optimal cut-off point of the PNI. A p-value of <0.05 was considered statistically significant. This study received ethical approval from the Ethics Committee of Ulin Regional Hospital under protocol number 25/KSM.Paru/Litbang/RSUDU/II/2023.

Results

This study included 976 hospitalized COVID-19 patients, consisting of 772 survivors and 204 non-survivors. Table 1 summarizes the patients' demographic, clinical, and laboratory characteristics. Non-survivors were significantly older than survivors ($p<0.001$), and male sex was also associated with increased mortality ($p=0.011$)Comorbidities such as hypertension ($p=0.004$), diabetes mellitus ($p=0.001$), and obesity ($p=0.036$) were more prevalent in non-survivors. Additionally, the number of comorbid conditions was significantly associated with mortality ($p<0.001$). Disease severity at admission was also linked to survival outcome ($p<0.001$). Laboratory results indicated that non-survivors had higher leukocyte counts (8700; 6555-11300), lymphocytopenia (10.5%; 7.1–15.5), lower albumin levels (3.2; 2.9-3.5), and lower PNI ratios (33; 37.1–41.1), all with p-values <0.001 (Table 1).

Table 1 Baseline Demographic, Clinical, and Laboratory Characteristics of COVID-19 Patients on Admission by Survival Outcome

Variable	All patients (n=976)	Survivors (n=772)	Non-Survivors (n=204)	p-value
Age, median (range), year	54 (44–62)	52 (43–60)	58 (49–65)	<0.001*
Male	54 (44–62)	53 (43–61)	60 (49–66)	<0.001*
Female	53 (43–61)	52 (42–60)	57 (49–64)	0.001*
Sex, no (%)				
Male	540 (55.3)	411 (42.1)	129 (13.2)	0.011α
Female	436 (44.7)	361 (37.0)	75 (7.7)	
Co-morbidities, no (%)				
Hypertension	443 (45.4)	332 (34.0)	111 (11.4)	0.004α
Diabetes Mellitus	322 (33.0)	234 (24.0)	88 (9.0)	0.001α
Chronic Kidney Disease	101 (10.3)	74 (7.6)	27 (2.8)	0.128α
Cardiovascular disease	80 (8.2)	60 (6.1)	20 (2.1)	0.347α
Stroke	39 (4.0)	30 (3.1)	9 (0.9)	0.733α
Malignancy	7 (0.7)	5 (0.5)	2 (0.2)	0.641β
Hepatitis B	11 (1.1)	6 (0.6)	5 (0.5)	0.059β
Lung tuberculosis	33 (3.4)	24 (2.5)	9 (0.9)	0.360α
COPD	4 (0.4)	3 (0.3)	1 (0.1)	1.000β
Asthma	27 (2.8)	21 (2.2)	6 (0.6)	0.864α
Obesity	477 (48.9)	364 (37.3)	113 (11.6)	0.036α
Number of Co-morbidities, no (%)				
0	171 (17.5)	158 (16.2)	13 (1.3)	
1	330 (33.8)	262 (26.8)	68 (7.0)	<0.001α
2	260 (26.6)	202 (20.7)	58 (5.9)	
≥3	215 (22)	150 (15.4)	65 (6.7)	
The severity of illness, no (%)				
Mild	94 (9.6)	92 (9.4)	2 (0.2)	
Moderate	240 (24.6)	234 (24)	6 (0.6)	<0.001α
Severe	160 (16.4)	146 (15)	14 (1.4)	
Critically ill	482 (49.4)	300 (30.7)	182 (18.6)	
Body Mass Index, no (%)				
Underweight (<18.5)	21 (2.2)	18 (1.8)	3 (0.3)	
Normal (18.5–22.9)	267 (27.4)	214 (21.9)	53 (5.4)	0.148α
Overweight (23–24.9)	211 (21.6)	176 (18)	35 (3.6)	
Obesity (≥25)	477 (48.9)	364 (37.3)	113 (11.6)	
Leucocyte, median (range)	7600 (5700–9800)	7300 (5502–9300)	8700 (6555–11300)	<0.001*
Lymphocyte, median (range)	15.5 (9.72–23.3)	17 (11.2–25)	10.5 (7.1–15.5)	<0.001*
Albumin, median (range)	3.4(3–3.8)	3.5 (3.1–3.9)	3.2 (2.9–3.5)	<0.001*
PNI, median (range)	40.0 (35.6–45.1)	41.0 (36.0–46.2)	33 (37.1–41.1)	<0.001*

α=Pearson Chi square, β=Fisher exact test *Mann-Whitney test

Table 2 Univariate Logistic Regression Analysis of Laboratory Parameters Associated With COVID-19 Mortality

Variables	Univariate		
	Odds Ratio (OR)	95% CI	p-value
Leucocyte	1.000	1.000-1.000	<0.001
Lymphocyte	0.914	0.894-0.934	<0.001
Albumin	0.472	0.375-0.624	<0.001
Prognostic Nutritional Index	0.923	0.901-0.945	<0.001

Univariate logistic regression analysis (Table 2) showed that leukocyte count (OR 1.000, 95% CI 1.000–1.000), lymphocyte percentage (OR 0.914, 95% CI 0.894–0.934), albumin level (OR 0.472, 95% CI 0.375–0.624), and PNI (OR 0.923, 95% CI 0.901–0.945) were significantly associated with mortality (all p<0.001). Receiver operating characteristic (ROC) analysis of PNI (Figure 1) identified a cut-off value of ≤41.9, with a sensitivity of 44.9%, specificity of 82.4%, and area under the curve (AUC) of 0.666 (p<0.001), indicating moderate discriminatory ability for mortality prediction.

Following the cut-off result of PNI (<41.9), we further analyzed the data to identify any significant correlations (Table 3). Interestingly, we found that age and gender were strongly correlated with PNI (p<0.001). Moreover, our analysis also revealed that disease severity and the number of co-morbidities were significantly correlated with PNI (p<0.001 and p=0.032, respectively), with co-morbidities such as diabetes mellitus (p=0.016) and asthma (p=0.034) demonstrating a particularly strong correlation.

Discussion

Older adultstend to be hospitalized, require intensive care, or die from COVID-19. Immunosenescence occurs with aging. Aging increases inflammation. Inflammaging may also cause cytokine release syndrome, a hyperinflammatory response that can damage organs and cause death.[7]

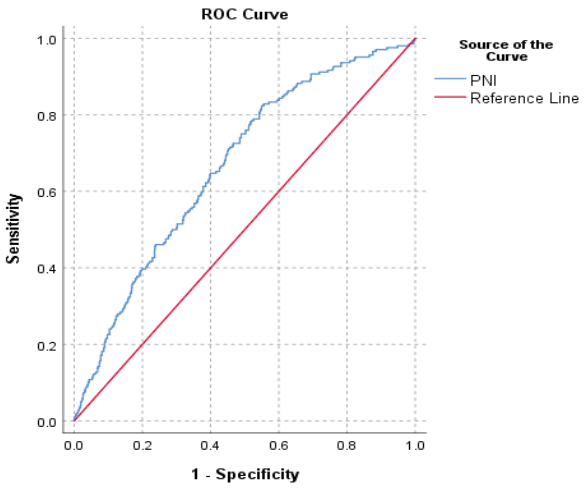

Figure 1 Receiver Operating Curve (ROC) for PNI
AUC = 0.666; cut-off ≤41.9; sensitivity 44.9%; specificity 82.4%; p<0.001

Table 3 Clinical Characteristics According to PNI

Variable	PNI≤41.9 (n=590)	PNI>41.9 (n=386)	p-value
Age, median (IQR), year	56 (48–64)	49 (37–58)	<0.001*
Male	57 (48–64)	49 (38–58)	<0.001*
Female	55 (48–62)	49 (36–58)	<0.001*
Sex, no (%)	590 (60.5)	386 (39.5)	<0.001α
Male	354 (36.3)	186 (19.1)	
Female	236 (24.2)	200 (20.5)	
Co-morbidities, no (%)			
Hypertension	277 (28.4)	166 (17.0)	0.226 α
Diabetes Mellitus	212 (21.7)	110 (11.3)	0.016 α
Chronic Kidney Disease	70 (7.2)	31 (3.2)	0.055 α
Cardiovascular disease	55 (5.6)	25 (2.6)	0.113 α
Stroke	24 (2.5)	15 (1.5)	0.887 α
Malignancy	6 (0.6)	1 (0.1)	0.255 β
Hepatitis B	8 (0.8)	3 (0.3)	0.541β
Lung tuberculosis	23 (2.4)	10 (1.0)	0.269α
COPD	3 (0.3)	1 (0.1)	1.000β
Asthma	11 (1.1)	16 (1.6)	0.034α
Obesity	280 (28.7)	197(20.2)	0.274 α
Number of Co-morbidities, no (%)			0.032α
0	88 (9.0)	83 (8.5)	
1	197 (20.2)	133 (13.6)	
2	169 (17.3)	91 (9.3)	
≥3	136 (13.9)	79 (8.1)	
The severity of illness, no (%)	590 (60.5)	386 (39.5)	<0.001α
Mild	24 (2.5)	70 (7.2)	
Moderate	84 (8.6)	156 (16.0)	
Severe	112 (11.5)	48 (4.9)	
Critically ill	370 (37.9)	112 (11.5)	
Body Mass Index			0.319α
Underweight (< 18.5)	16 (1.6)	5 (0.5)	
Normal (18.5–22.9)	168 (17.2)	99 (10.1)	
Overweight (23–24.9)	126 (12.9)	85 (8.7)	
Obesity (≥25)	280 (28.7)	197 (20.2)	
Leucocyte	8000 (6000–10.200)	7000 (5500–9100)	<0.001*
Lymphocyte	12 (8–17)	23 (15–31)	<0.001*
Albumin	3.1 (2.9–3.4)	3.9 (3.7–4.2)	<0.001*

α=Pearson Chi square, β=Fisher exact test, *=Mann -Whitney test

The present study observed that male patients exhibited a higher mortality rate, which aligns with prior research. Multiple factors may contribute to the increased mortality among COVID-19-positive males. It also may be related to the protective effects of estradiol, which has been demonstrated to boost immune responses to viral infections.[8] Lastly, behavioral factors, such as higher smoking rates and reduced mask-wearing and social distancing, may contribute to the higher mortality rate in males with COVID-19.

This study found that pre-existing conditions like hypertension, diabetes and obesity may need more intensive treatment to survive. As with previous study, this comorbidity has been identified as a significant risk factor for COVID-19-related mortality. [9] There are several possible mechanisms between diabetes mellitus and mortality. Uncontrolled hyperglycemia increases pro-inflammatory cytokines and decreases anti-inflammatory cytokines, which can enhance COVID-19-related inflammation. In regard to hypertension, the pathophysiology underlying the relationship between hypertension and COVID-19 mortality is not fully understood. Nevertheless, the renin-angiotensin-aldosterone system (RAAS) may affect COVID-19 severity. SARS-CoV-2, which produces COVID-19, penetrates cells through the angiotensin-converting enzyme 2 (ACE2) receptor, widely expressed in the lungs, heart, and kidneys. ACE2 controls the RAAS, which controls blood pressure. SARS-CoV-2 may overactivate the RAAS, causing hypertension.[10]

In obesity, adipose tissue's participation in inflammation and immunological response underlies the obesity-COVID-19 mortality. Inflammatory cytokines and adipokines, such as leptin, are produced by adipose tissue and decrease immunological function. Insulin resistance and obesity can cause persistent inflammation and immunological dysfunction. Obesity also reduces lung capacity and respiratory muscle strength, making breathing and recovering from respiratory illnesses difficult.[11] COVID-19 mortality is also strongly correlated with the number of co-morbidities. United Kingdom studies found COVID-19 individuals with two or more co-morbidities were found more likely to die.[12] In terms of disease severity, we also found it correlates with mortality. Many studies have linked COVID-19 illness severity to death. In a comprehensive review and meta-analysis of 46 studies, Chaudhry et al. observed that severe COVID-19 had a greater mortality rate than severity and mortality.[13]

In contrast, body mass index (BMI) did not significantly predict mortality in this study. This finding reflects inconsistencies in the literature, with some studies reporting associations between elevated BMI and mortality, while others do not. A meta-analysis by Klanget al. [14] involving over 149,000 patients, similarly found no strong relationship between BMI and mortality. This suggests thatBMI may not significantly predict mortality in COVID-19 patients. However, it is essential to note that BMI is just one aspect of body composition and does not necessarily reflect overall health status. Further research is needed to understand the potential association between BMI and other COVID-19 outcomes, such as disease severity and hospitalization rates.[14]

Leucocyte, lymphocyte, albumin, and PNI were measured. In all analyses, survivors exhibited greater median ranges than non-survivors ($p<0.001$). In the univariate study, leucocyte, lymphocyte, albumin, and PNI had significant relationships with survival ($p<0.001$), suggesting they may be good COVID-19 prognostic markers. Leucocyte, lymphocyte, and albumin demonstrated mortality correlation ($p<0.001$). PNI also correlated with death, suggesting that these parameters may be valuable prognostic indicators for COVID-19 patients, with similar results in univariate analysis. An Italian study found that COVID-19 patients who died had greater leukocyte counts than survivors. Leukocytosis in COVID-19 may signify immunological activation and inflammation. In severe COVID-19 cases, immune activation may cause cytokine storms that damage tissues and organs. Leukocytosis also increases the risk of thrombotic events, which can cause death in severe COVID-19 patients.[15]

Lymphocytes also have a role in COVID-19 immunity, and their numbers correlate with disease severity and mortality. According to several studies, prevalence and impact of lymphopenia, or low lymphocyte counts is frequent in severe COVID-19 patients and associated with poor clinical outcomes. In Wuhan, China, COVID-19 non-survivors had higher lymphopenia than survivors. Severe cases had much lower lymphocyte counts than moderate cases.[16] It is believed that the depletion of lymphocytes in COVID-19 may be due to the virus's ability to directly infect and destroy these cells, as well as the cytokine storm that can occur in severe cases, leading to immune dysregulation and lymphocyte apoptosis.[17]

Low plasma albumin levels are linked to

COVID-19 mortality. Albumin governs cell entry, maintains intravascular fluid balance, and binds medicines and other substances. It is a practical laboratory measure used to assess malnutrition. Inflammatory cytokines inhibit albumin production, a negative acute phase reactant. Inflammation lowers albumin. The reasons behind low albumin levels in COVID-19 are not yet fully understood. The liver produces albumin and circulates for 21 days. However, previous and current studies have found that severe cases of COVID-19 are more likely to have low levels of albumin compared to mild cases. This cannot be solely explained by liver dysfunction due to damaged liver cells, as the onset of hypoalbuminemia is much quicker than it takes for albumin to break down in the body. Therefore, it is unlikely that severe COVID-19 leads to decreased albumin production.[18]

As a computed inflammatory index of hypo albumin and lymphopenia conditions in patients, this study conducted an analysis of the PNI value as a predictor of COVID-19 mortality with a cut-off value of PNI<41.9. From the characteristic analysis of the cut-off value of PNI, disease severity and co-morbidities were found to be significantly correlated with PNI. The co-morbidities related to PNI are diabetes mellitus and asthma. Notably, no correlation was found between BMI and PNI, emphasizing the importance of considering multiple factors in assessing an individual's overall health status. In addition, our analysis revealed that several biomarkers, including leucocytes, lymphocytes, and albumin, were strongly correlated with PNI, providing further evidence of the potential clinical utility of these markers in assessing an individual's nutritional and immunological status. These findings have important implications for managing patients with various co-morbidities, highlighting the need for a comprehensive approach that considers a wide range of factors.

This study also found that PNI correlates with mortality. Fever, respiratory muscular exertion, and endocrinological disturbances that increase gluconeogenesis, protein breakdown, and lipid oxidation contribute to COVID-19-related malnutrition.[19] A recent study found that PNI significantly predicted ICU admission and mortality in COVID-19 patients.[20] In this study, a PNI cut-off value of <41.9 was significantly associated with mortality, comorbidities (notably diabetes and asthma), and disease severity. These results are in line with prior research. Studies by Aciksari et al.,[21] Cakirca et al.,[22] and Kosovali et al.[23] have each demonstrated similar PNI thresholds correlating with increased mortality in COVID-19 patients. In addition, Doganci et al. found that patients with a higher PNI (>44.7) were in the survivor group.[24]

The study also examines the relationship between PNI and several other variables, including age, gender, diabetes mellitus, asthma, number of co-morbidities, and disease severity. Hung et al.'s systematic review and meta-analysis supported the use of PNI as a predictor of mortality with an aggregated sensitivity of 0.76 and specificity of 0.71 (AUC of ROC: 0.79) but also revealed a negative correlation between PNI and disease severity in COVID-19 patients. In hospitalized COVID-19 patients, a low PNI was associated with a sevenfold increase in mortality risk, as demonstrated by their findings. The last one is the correlation between PNI and disease severity with several research findings indicating that PNI correlates with severity and can be used as a predictor.[25,26]

This study has several limitations. First, it is a single-center study, which means the findings may not be generalizable to all COVID-19 patients, as the cases included were likely severe or critical. Second, selection bias may have influenced the results, as only hospitalized patients were included in the study. Additionally, this retrospective study relied on data available from the patients' medical records during the first 48 hours of admission, including anthropometric measurements (weight and height). The vaccination status of the patients was not included in the study.

In conclusion, the prognostic nutritional index (PNI) is a simple and inexpensive method that may be quickly computed using common laboratory values. The results suggest that the PNI, which reflects the patients' immune system and nutritional status upon hospital admission, can be a reliable predictor of in-hospital mortality COVID-19 patients.

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Acute Toxicity (LD_{50} value) of Peppermint (Mentha piperita) Suspension in Female Mice

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Abstract

Peppermint has several benefits, such as antibacterial, anti-inflammatory, and antifungal properties, that leads to its use in various products, especially in herbal medicine. This study aimed to evaluate the LD50 value of peppermint suspension through an acute toxicity test. This study was conducted at the Animal Laboratory Management Unit of the School of Veterinary Medicine and Biomedical Sciences, IPB University, from October 9 to 30, 2024. A total of 25 female mice were divided into 5 groups, and given different doses of peppermint suspension of 0, 0.5, 1, 1.5, and 2.0 g/kgBW. Observations were then carried out for 14 days on the parameters of body weight, physiological responses, clinical symptoms, absolute and relative organ weight, body weight, and number of mortalities per day. Results showed that the highest mortality was found in the groups administered with a 1.5 and 2.0 g/kgBW of peppermint suspension. Clinical symptoms, such as hair standing, lethargies, and decreased locomotor activity were also observed in these groups. In addition, the administration of peppermint suspension in mice had no significant effect on body weight, as well as on absolute and relative organ weight, It was also demonstrated that organs collected did not present any significant lesion. Thus, the acute toxicity test of peppermint suspension showed no macroscopic lesion or changes in organs and body weight. The LD50 value suggested that the is in the moderate toxicity category, with an LD50 value of 1.92 g/kgBW.

Keywords: Acute toxicity, alternative medicine, herbal extract, mice, peppermint

Introduction

Indonesia is a country abundant in natural resources, including medicinal plants. It is estimated that over 30,000 plant species exist in the country, with approximately 7,000 identified as medicinal.[1] The benefits of these medicinal plants have been studied, used in daily lives, inherited by the ancestors, and preserved by the community. In addition, various studies are competing to prove their efficacy, leading to the high potential of the herbal medicine industry. One of the medicinal plants that is often used in the industry is peppermint leaves.

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Peppermint plant belongs to the Lamiaceae family, which has approximately 30 species and various hybrids. It generally grows in sub-tropical areas,[2] and the leaves are recognized for their antineoplastic, antibacterial, anti-inflammatory, antiallergic, antifungal, antihepatotoxic, antiviral, antiradiation, and antinociceptive properties.[3] The main components of peppermint identified through hydro distillation are menthol (45.34%), menthone (16.04%), menthofuran (8.91%), cis-carane (8.70%), 1,8-cineole (4.46%), neo-menthol (4.24%), and limonene (2.22%).[4] These components give a soothing aroma, cool taste, and a variety of benefits for the body, leading to the wide use of the leaves in commercial medicines, toothpaste, aromatherapy, and food and beverage flavors. Peppermint has also been commonly used in some certain regions of Indonesia as an addition to herbal medicine.

Several studies have examined the benefits

of using peppermint, with some reporting that taking 40 drops of commercial peppermint extract in complementary medicine can improve nausea, vomiting, and anorexia in breast cancer patients undergoing chemotherapy.[5] Although peppermint leaves have many benefits for the body, their usage needs to be limited. The high menthol component causes side effects when consumed in excessive amounts, including agitation, dizziness, ataxia, hallucinations, seizures, and coma.[6] An acute toxicity test can be conducted to test the safety limit of consuming peppermint leaves to ensure that any consumable preparations made using mint use the safety limit as a reference. This test has the potential to identify the toxic effects of a preparation in a short period and is given through the oral route. Previous studies had been conducted regarding the toxicity limit of peppermint, such as Malekmohammad et al.[7] who observed the toxic effect of peppermint essential oil on mice, humans, insects, and rabbits. A study conducted by Yousuf et al.[8] also observed the acute oral toxicity of Japanese mint oil towards brine shrimp. Despite the existing literature, none has examined the LD_{50} of peppermint extract suspension on mice, along with observation of macroscopic organ lesion, organ weights, or body weight changes. Therefore, this study aims to determine the LD_{50} value and the effect of peppermint suspension on organ weight, macroscopic organ lesions, and body weight in female mice.

Methods

This study was approved by the Animal Ethics Commission of the School of Veterinary Medicine and Biomedicine, IPB University, with approval number 098/KEH/SKE/VIII/2023. The research was conducted at theAnimal Laboratory Management Unit, School of Veterinary Medicine and Biomedical Sciences, IPB University, from October 9 to October 30, 2023

The tools used in this study were 5 mice cages made of plastic, with dimensions of 35 cm, 25 cm, 10 cm, wire cover cages, using wood shavings as bedding. Each cage was set with a drinking bottle of 80 mL, digital scales, syringes, micropipettes with a capacity of 0 to 100 µ, digital thermogenic, and minor surgical tools. The materials used were commercially dried peppermint leaves obtained from Gubuk Herbal, Solo Regency, Indonesia, distilled water, and 25 *Deutschland Denken Yoken* (DDY) female mice weighing 20 to 30 g. Ivermectin was administered as an anthelmintic for 7 days in a row during acclimatization to ensure that the mice were free from any parasite infestations, preventing any potential interference with the study parameters.

Preparation for the peppermint suspension was performed by boiling 500 grams of dried peppermint leaves in 1000 mL of water until a full boil was reached, then maintaining the boil for 15 minutes. Subsequently, the dried peppermint leaves were filtered using a strainer.

The preparation of experimental animals and cages began with the acclimatization process. In this study, the initial stage started with cleaning the cage and preparing the tools that were used during the treatment period. The animals were acclimatized for 7 days to ensure that the mice could adapt to the new cage conditions and reduce the possible stress level. During the acclimatization process, mice were fed with 10% of their body weight and provided with ad libitum drinking water. Anthelmintic preparations were administered, which was ivermectin (0.04 mg/kg, diluted with distilled water), once a day for 7 days. Replacement of cage bedding in the form of wood shavings was done every 7 days.

This study was designed according to the Completely Randomized Design (CRD) method. An acute toxicity study was performed according to the method described by BPOM (2020), with modifications. The mice were separated into 5 groups based on the dosage administered. Each group contained 5 mice, with a negative control group and treatment groups administered with peppermint suspension in the dosage of 0.5, 1, 1.5, and 2.0 g/kgBW, respectively. The volume administered were 0.03, 0.06, 0.09, and 0,12 mL, respectively. Treatment was conducted by administering dried peppermint leaves suspension, which was purchased commercially and prepared by boiling it in water. Furthermore, the peppermint suspension was administered only once on the first day.

Observations during the study were performed every day after treatment on day 0 until the next 14 days. Parameters used for observation comprised the body weight, physiological response, clinical symptoms, absolute and relative organ weight, and the number of mortalities per day. Clinical symptoms observed included behavior, appetite and drinking, defecation, urination, hypersalivation, tremors, convulsions, and paralysis. Furthermore, physiological responses observed were body temperature, respiration

rate, and heart rate rate. Body weight weighing of mice was conducted once every 7 days, on days 0, 7, and 14 post-treatments. The experimental parameter to determine the value of LD_{50} in the acute toxicity test was to observe the number of mortalities of mice in each group from day 0 to day 14. Mice that died was later dissected for organ collection and weighing. Mice that were still alive until day 14 were euthanized by the cervical dislocation method. Subsequently, the removal of organs in the body (liver, innards, kidneys, lungs, heart, spleen) was performed for macroscopic observation of the organs and organ weighing using digital scales. The relative weight of the organ was obtained by the dividing value of the absolute weight of the organ by the body weight of the mice on the last day of observation and then multiplied by 100%.[10] The organs were also examined macroscopically for any signs of abnormalities.

Data were analyzed using Kruskall-Walli's method from the Minitab 19 software and the value of LD_{50} was measured using Probit analysis. The probit model assumed that the relationship between the independent variables and the probability of the outcome was determined by the Cumulative Distribution Function (CDF) of the normal distribution. This was different from logistic regression, which used the logistic (sigmoid) function. The data from the analysis and LD_{50} value could be interpreted to continue the discussion of the study results.

Results

The major parameter observed in acute toxicity testing (LD_{50}) to determine the toxicity level of the peppermint suspension was the percentage of mortality or death.[11] Mortality rate was analyzed from the day of peppermint suspension administration to day 14 using the probit analysis method. Mortality was observed at the dosage of 2.0 g/kg BW on day 3 and at the dosage of 1.5 g/kg BW on day 10. The result of the analysis of the LD_{50} value of peppermint suspension obtained using the probit analysis method was 1.92 g/kg BW. The mortality rate of mice at several dosages of peppermint suspension was presented in Table 1.

Observation of clinical symptoms in mice was routinely monitored once in a day at the same hour for 14 days after the administration of peppermint suspension. Based on qualitative observations, abnormal clinical symptoms were found in several mice, such as in the mice administered with dosages of 1.5 and 2.0 g/kgBW. The clinical symptoms shown by several mice were symptoms of depression, hair standing, lethargies, and decreased locomotor activity.

Other parameters observed were physiological responses and the body weight of the mice. Physiological responses observed were heart rate, respiratory rate, and temperature, which were observed routinely once a day at the same hour, starting from day 0 to day 14, while body weight weighing was conducted on days 0, 7, and 14 after mint suspension administration. The results of data analysis of body weight and physiological responses of mice were displayed in Figure 1.

Based on Figure 1, the results of the analysis of the average body weight using Kruskall-Wallis's method did not indicate any significant differences in the administration of peppermint suspension in various doses compared to the control (A). This proved that the administration of mint suspension did not affect the body weight of mice.

In this study, the parameter of heart rate was shown in Figure 1. The average results of heart rate were in a fluctuating range, which was 86 to 216 ×/minute (B and the average respiratory rate was also fluctuating, and was in the range of 82-147 ×/minute (C). Furthermore, the average

Table 1 Relationship Between Peppermint Suspension Dosage and Mortality Rate in Mice

Peppermint Suspension Dosages (g/kgBW)	Number of Mice	Number of Mortalities	Day of Mortality	Mortality Rate (%)
0	5	0	-	0
0.5	5	0	-	0
1	5	0	-	0
1.5	5	1	10	20
2	5	1	3	20

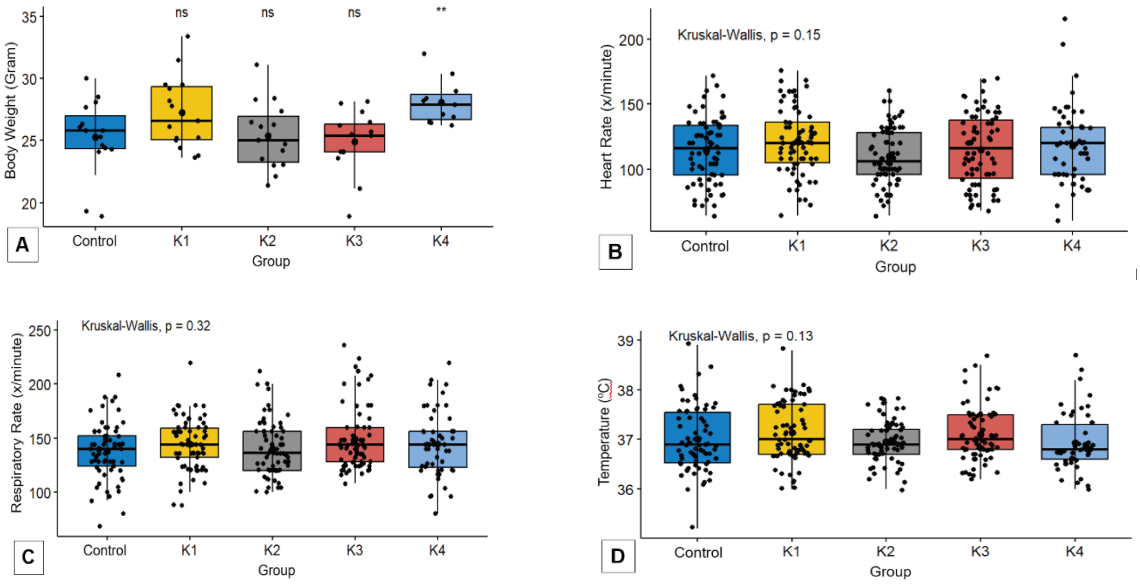

Figure 1 Peppermint Suspension Effect on Body Weight and Physiological Responses of Mice

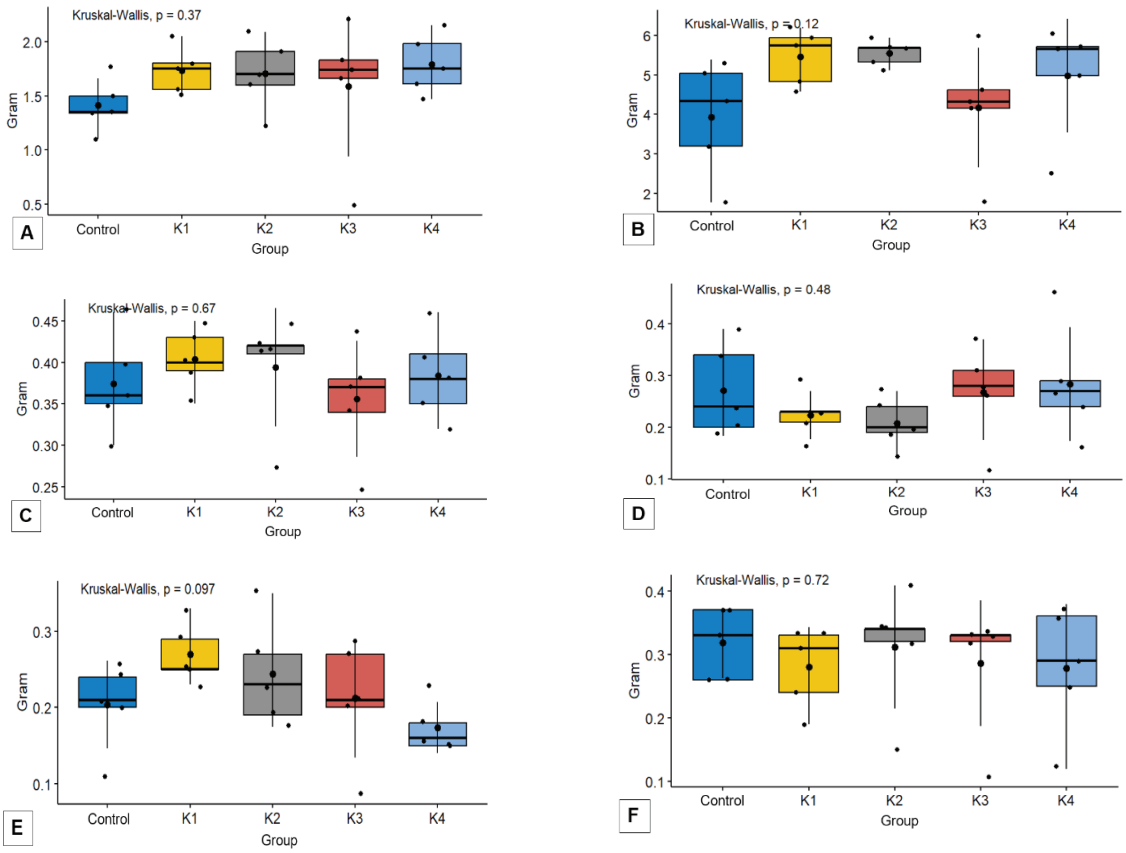

Figure 2 Effect of Peppermint Suspension on Absolute Organ Weights of Mice
(A) Liver; (B) Intestines; (C) Kidneys; (D) Lungs; (E) Heart; (F) Spleen

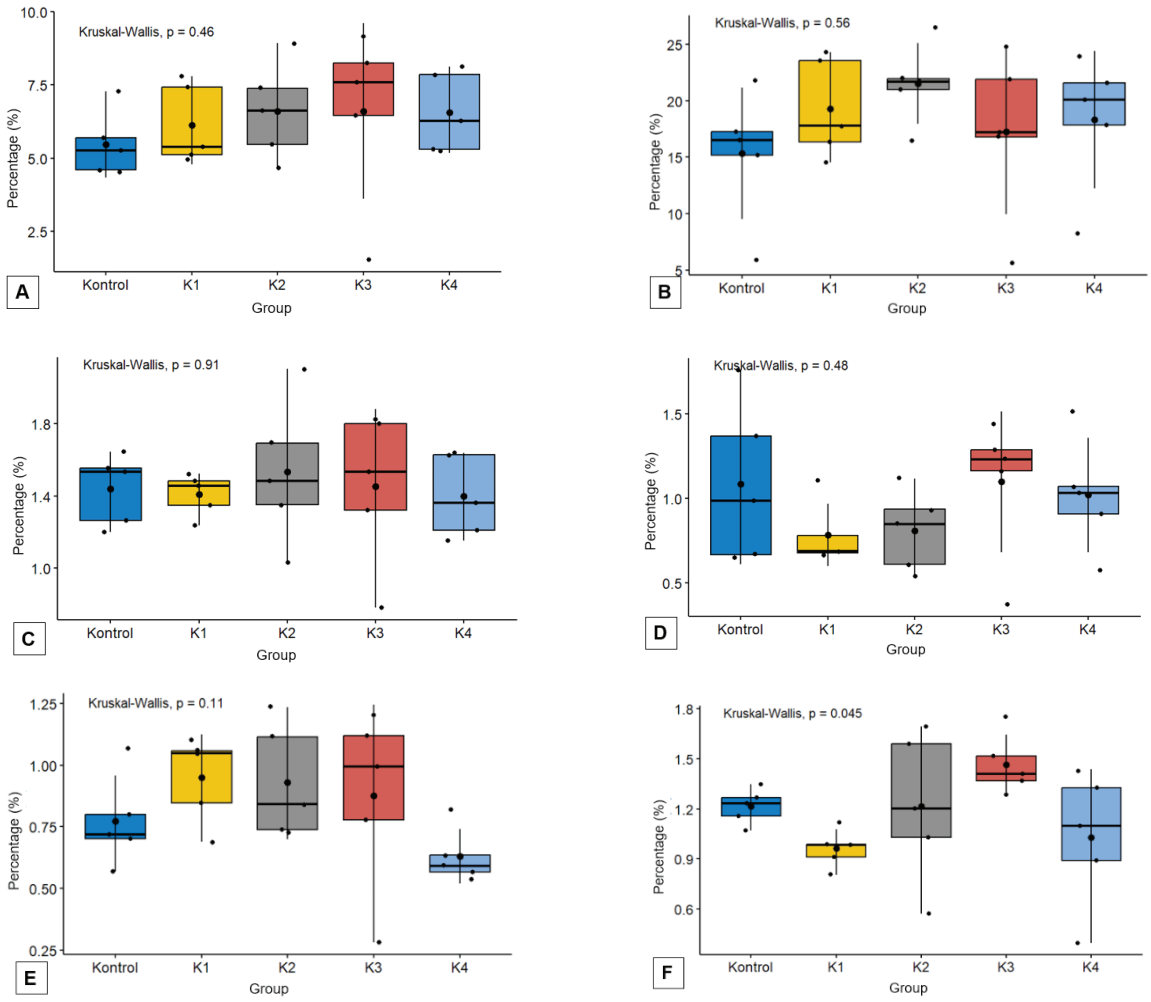

Figure 3 Effect of Peppermint Suspensionon Relative Organ Weights of Mice (A) Liver, (B) Innards (C) Kidneys (D) Lungs (E) Heart (F) Spleen

temperature results were in the range of 36.3 to 38.5°C (D). The average heart rate, respiration rate, and temperature of mice for 14 days since the administration did not reveal any significant difference between mice administered with peppermint suspension in various dosages compared to the control. This proved that peppermint suspension did not influence the physiological responses of mice in the form of heart rate, respiratory rate, and temperature observed.

Macroscopic observation of the organs indicated that all organs were normal in color, with the heart, liver, and spleen showing a reddish-brown color, the innards showing a pinkish-pale color, and the lungs showing a pale pink color. No lesions, nodules, hemorrhage, or fatty deposits were observed.

According to Figure 2, there was no significant difference found in the absolute weight parameters of liver (A), innards (B), kidneys (C), lungs (D), heart (E), and spleen (F) between mice administered with peppermint suspension at various dosages and control. This proved that the administration of mint preparations at doses of 0.5, 1, 1.5, and 2.0 g/kg BW did not affect the absolute organ weight.

Similarly, Figure 3 shows that the relative weights of the same organs did not significantly differ across treatment groups. These findings support the conclusion that peppermint suspension, at doses up to 2.0 g/kg BW, did not affect relative organ weight.

Discussion

Peppermint was one of the traditional herbal plants that was widely used in Indonesia[9], it had a high menthol content, which accelerated circulation, and relieved bloating, nausea, and cramps. Furthermore, peppermint also exhibited secondary metabolite compounds, namely tannins and flavonoids, which were believed to accelerate the digestive system.[10,11] Based on several studies, mint leaves were shown to have high antioxidant content, antimicrobial, antitumor, and antialergenic.[12]

Acute toxicity tests were conducted to examine toxic effects occurring within a short time after administration of a substance. Generally, acute toxicity tests were carried out within at least 24 hours.[13] Determination of the lethal or toxic dose range was expressed as LD_{50}.[14] When a suspension did not cause mortality up to 14 days post-treatment, then the suspension could be categorized as a practically non-toxic suspension.[15] The LD_{50} value calculated by the probit analysis method was obtained as 1.92 g/kg BW. Furthermore, those in the range of 0.5 to 5 g/kgBW were categorized as moderate toxicity.[16]

Parameters of mortality that could be observed in mice administered with dosages of 1.5 g/kg BW and 2.0 g/kgBW were suspected of causing damage to the cerebellum in mice. The content of menthone and menthofuran in mice was proven to cause an increase in liver weight, hepatotoxicity, and damage to the cerebellum in a short time.[7] This damage was reinforced by clinical symptoms displayed in mice, such as the presence of incoordination, symptoms of depression, and decreased locomotor activity. In this study, the cerebellum was the part of the brain that controlled balance, orientation, body positioning, muscle tone, and coordination.[17] This was consistent with the findings of clinical symptoms in mice administered with dosages of 1.5 g/kgBW and 2.0 g/kgBW, including depression, lethargies, and decreased locomotor activity. However, an increase in liver weight was not observed. This was likely because a higher dosage could be required for such an effect to occur.

Observations of clinical symptoms on acute toxicity test of peppermint suspension indicated abnormalities in clinical symptoms at doses of 1.5 g/kgBW and 2.0 g/kgBW. Clinical symptoms that appeared were depression, standing hair, lethargies, and decreased locomotor activity. According to previous studies, peppermint was known to contain components such as menthol (45.34%), menthon (16.04%), menthofuran (8.91%), cis-carane (8.70%), 1,8-cineole (9.45%), neo-menthol (4.24%), and limonene (2.22%). Furthermore, the content of menthol isomers in mint (L-menthol, D-menthol, and D/L-menthol) in excessive administration could irritate the eyes, skin, respiratory system, and coordination system.[7] This could also result in contributing to the clinical symptoms that occurred during the study, besides the suspected cerebellum damage caused by methon and methofurane.

Toxicity could affect physiological response through several mechanisms, with effects ranging from mild to severe, depending on factors such as dose, exposure duration, and specific substances involved. Several pathways contributed to these effects, including the disruption of cellular homeostasis, slowly leading to the decline of physiological parameters like temperature, heart rate, and respiratory rate.[18] Toxicity could also trigger an inflammatory response by activating immune cells,[19] potentially leading to increased body temperature. Furthermore, when the toxic substance induced neurotoxicity, it could alter neurotransmitter levels, damage neurons, or interfere with ion channels,[20] causing abnormality in heart and respiratory rate. In this study, the physiological response parameters of the mice, such as heart rate, respiratory rate, and temperature, showed no abnormalities. The heart rate of the mice was in the range of 86 to 216 ×/min, which was still in the normal range, showing 85 to 216 ×/minute.[21] Respiratory rate observed after administration was in the range of 82 to 147 ×/minute, which was still in the normal respiratory rate of mice, indicating 80 to 230 ×/minute.[22] The temperature of the mice was in the range of 36.3 to 38.5°C, which was below the normal range, ranging from 36.5 to 38.0°C.[21] The temperature difference was influenced by room temperature and differences in the metabolic rate of the mice's body.

The body weight parameters of the mice did not show any significant increase or decrease, and there was no difference between the body weight of the control and the mice given peppermint suspension at several dosages. Subsequently, the body weight of mice in the treatment group was still within the normal weight range, which was 18 to 35 grams.[23]

Calculation of absolute weights and macroscopic observations of mice organs were conducted as an indicator of the presence or absence of toxic effects of a compound on the organs of experimental animals. Based on the recommendations of The Society of Toxicology

Pathology (STP), the organs that were used as indicators of acute toxicity tests were the liver, kidneys, spleen, lungs, and heart.[24] This was due to the close ties between these organs in the metabolic and detoxification processes. Macroscopic observations were made to observe whether there was damage to the organs. Damage observed such as changes in liver color to yellow-brown, cell degeneration, and the discovery of masses that indicated inflammation, either chronic or acute inflammation, abscesses, and malignant tumors.[25] Based on Figure 2, the administration of peppermint suspension did not result in any macroscopic changes in these organs or any differences in organ weights between control mice and mice given peppermint suspension administration in various doses. This proved that the administration of mint suspension did not influence any of the observed organs.

Absolute organ weight was one of the parameters that provided accurate results in toxicity testing of a substance to target organs, however, absolute weight measurement was often followed by relative organ weight measurement as the correlation was strong. Relative weight referred to the ratio between organ weight and animal body weight.[26] As a sensitive indicator of organ damage, changes in organ weight were difficult to interpret.[27] This could be caused by organ weight being affected by body weight, causing variation due to differences in body weight. Relative organ weights were analyzed for the same organs, namely the liver, innards, kidneys, lungs, heart, and spleen, which did not indicate any significant differences.

Based on the findings on the parameters, administration of peppermint suspension caused mortalities on the dosage of 1.5 g/kgBW and 2 g/kgBW. Mice administered with the dosage of 1.5 g/kgBW and 2 g/kgBW also revealed clinical signs such as depression, lethargy, standing hair, and decreased locomotor activity. However, no macroscopic lesions were shown on the organs observed, which were liver, innards, kidneys, lungs, heart, and spleen. No significant difference was displayed in physiological responses, body weight, and absolute and relative organ weight. According to the number of mortalities, the LD_{50} value of this study was found at 1.92 g/kgBW. Despite no macroscopic lesions shown, and no significant difference on any other parameter, the LD_{50} value showed that peppermint suspension administration in mice was in the moderate toxicity category. Based on the Hodge and Sterner test of toxicity (BPOM 2014)[15], the LD_{50} value of the moderate toxicity category was 0.5 to 5 g/kgBW. Compared to another study conducted by Malek et al.[7] the administration of peppermint oil towards mice proved that peppermint oil also fell into the category of moderately toxic (≤2 g/kgBW) towards mice, with an LD_{50} value of 1.6 g/kg BW. In another study conducted by Yousuf et al.,[8] acute toxicity of Japanese mint oil on brine shrimp indicated an LD_{50} value of 2070.4 µl/kg and was in the moderate category.

According to these findings, peppermint suspension administration on mice was in the category of moderate toxicity based on the number of mortalities and the clinical signs shown by the group administrated with a dosage of 1.5 g/kgBW and 2 g/kgBW, with the LD_{50} value of 1.92 g/kgBW. This suggested that administration above 1.92 g/kgBW of peppermint suspension could cause death or clinical symptoms such as depression, lethargy, and decreased locomotor activity. Furthermore, this study did not include microscopic observation of the organs, which could show any significant lesion that occurred microscopically toward the organs. Further studies must be carried out including microscopic observation of the organs, such as histopathology, to determine whether the suspension could affect organs microscopically.

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Local Tumor Control Affects Survival of Patients with Osteosarcoma and Ewing Sarcoma

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Abstract

Osteosarcoma and Ewing sarcoma are the most common primary malignant bone tumors in children. This study aimed to analyze the characteristics of osteosarcoma and Ewing sarcoma patients at Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, from 2020 to 2023 and compare the one-year survival rates between patients who underwent complete treatment (surgery for local tumor control and chemotherapy) and those who received chemotherapy alone. This study used a descriptive-analytical observational design with a retrospective approach by reviewing electronic medical records from the Indonesian Pediatric Cancer Registry (IPCAR). A total of 44 osteosarcoma patients and 14 Ewing sarcoma patients who met the inclusion criteria were included. The majority of patients were boys (osteosarcoma=61.3%; Ewing sarcoma=64.3%), with a mean age of 13.5 years for osteosarcoma and 9.1 years for Ewing sarcoma. Osteosarcoma was most commonly found in the femur and tibia (90.9%), whereas Ewing sarcoma was primarily located in the axial region (71.4%). Most patients underwent chemotherapy without local tumor control (osteosarcoma=61.4%; Ewing sarcoma=57.1%). The analysis revealed a significant difference in one-year survival between patients who received local tumor control combined with chemotherapy and those who underwent chemotherapy alone (osteosarcoma p=0.000; Ewing sarcoma p=0.010). In conclusion, local tumor control significantly improves one-year survival in both tumor types.

Keywords: Chemotherapy, Ewing sarcoma, osteosarcoma

Introduction

Bone tumors can be classified as either malignant or benign. A primary malignant bone tumor originates from primitive mesenchymal cells, while a secondary bone tumor originates in other tissues and metastases to the bone. Primary bone tumors contribute to 0.2% of the world's malignancy rate, with many cases exhibiting idiopathic causes. There are several types of primary malignant bone tumors, including osteosarcoma, Ewing sarcoma, and chondrosarcoma. Osteosarcoma is the most common primary bone malignancy in pediatrics, while Ewing sarcoma is the second most common malignant bone tumor, accounting for 10-15% of all malignant bone tumors. It primarily affects children during adolescence.[1–3]

Osteosarcoma exhibits a bimodal age distribution. The first peak is observed in children aged 10-14 years, corresponding to pubertal growth, while the second peak is observed in adults aged >65 years. Furthermore, boys are more commonly affected than girls. At initial diagnosis, approximately 15-20% of patients present with lung metastases, and 40% of patients develop metastases at a later stage. Ewing sarcoma represents the second most common bone malignancy in children, with 90% of cases occurring between the ages of 5 and 25. Like in osteosarcoma, boys are also more commonly affected than girls in Ewing sarcoma.[3–5]

Several studies have been concerned with identifying the causes of osteosarcoma by examining factors such as genetics, epidemiology,

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and the environment. Genetic-related conditions include patients with hereditary retinoblastoma, which is associated with loss of heterozygosity of the RB gene; Li-Fraumeni syndrome, which is associated with lineage mutations of the P53 gene; and Rothmund-Thompson syndrome, which is due to mutations in the RECQL4 gene. Osteosarcoma patients frequently present with symptoms including pain hobbled walking, and an increasing lump on the affected limb over several months. Patients typically seek treatment following a trauma or high-intensity physical activity. On physical examination, there may be limited range of motion, joint effusion, pain on pressing, and the limb may feel warmer. Meanwhile, examination with X-rays typically reveals a sunburst pattern.[3,4]

Risk factors for Ewing sarcoma include parental exposure to pesticides or chemical solvents and increased maternal and paternal age at conception. Approximately 95% of Ewing sarcoma cases are estimated to be caused by abnormalities in mesenchymal progenitor cells. This is triggered by genetic factors, whereby Ewing sarcoma patients exhibit a distinctive chromosomal rearrangement in the form of a non-random translocation, specifically t(11;22) (q24;q12) or t(21;22)(q22; q12), which results in aberrant transcription and coding. Moreover, this mutation increases the cell fusion activity of proteins from the Ewing sarcoma (EWS) gene on chromosome 22, the Friend Leukemia Integration-1 (FLI-1) gene on chromosome 11, or the Erythroblast transformation-specific Related Gene (ERG) gene on chromosome 21. The symptoms of Ewing sarcoma typically manifest as pain that worsens at night, stiffness, and swelling that persists over weeks to months. These symptoms are often accompanied by the development of progressively larger bone lesions. Due to the unspecific symptoms, malignancy can only be diagnosed when the onset has been running for three to nine months, which may delay the timing of appropriate treatment. On radiological examination, the characteristic appearance of an onion peel may be observed, which indicates a periosteal reaction, a common feature of Ewing sarcoma.[3,6,7]

The standard treatment for these malignancies consists of a multimodal approach, including surgery for local tumor control and chemotherapy for systemic disease control. Surgery plays a crucial role in removing the primary tumor and improving survival outcomes. Meanwhile, chemotherapy is essential in eradicating micrometastatic disease and reducing the risk of recurrence. Based on the data in Dr. Hasan Sadikin General Hospital Bandung, despite the established treatment guidelines, 60% patients refuse surgical intervention due to concerns about postoperative morbidity, functional impairment, or financial difficulty. As a result, some patients undergo chemotherapy as their sole treatment modality, potentially affecting their overall prognosis.

Patients with bone tumors experience limitations in performing daily activities and a decrease in physiological function. This shows an urgent problem to decrease the morbidity and mortality rate of childhood malignant bone tumors. One of the initial steps to achieve this is to reveal the real characteristics of childhood malignant bone tumor patients. The incidence of bone tumors has been observed to be higher in certain demographic groups. This study identifies patient characteristics, including age, gender, laterality, histopathological pattern, primary tumor location, treatment, and one-year survival. This study aims to describe the clinical features, histopathological patterns, treatment modalities, and one-year survival outcomes of pediatric patients with osteosarcoma and Ewing sarcoma. It also compares survival rates between patients receiving complete treatment (surgery and chemotherapy) and those receiving chemotherapy alone at Dr. Hasan Sadikin General Hospital, a tertiary referral center in West Java.

Methods

This study was a descriptive analytical observational study using secondary data from electronic medical records available on the Indonesian Pediatric Cancer Registry (IPCAR) website. Patient data were filtered by diagnosis codes for osteosarcoma and Ewing sarcoma, then exported into Microsoft Excel format. Several researchers reviewed the exported data based on the inclusion criteria, namely patients diagnosed with osteosarcoma and Ewing sarcoma at Dr. Hasan Sadikin General Hospital Bandung from 2020 to 2023. The exclusion criteria were patients with incomplete medical record data, patients who did not undergo treatment, and patients lost to follow-up. For this study, a total of 58 research subjects were included. After all the data is collected, the data editing, coding, and verification processes are carried out. Data analysis was done with Stata/SE 16. Analytical and descriptive data were analyzed. Categorical scale information, including gender, age,

laterality, treatment, histopathological pattern, primary tumor location, and one-year survival. All of the data are presented as percentages and frequency distributions. Because more than 20% of the cells have an expected count of less than 5, the Fisher exact test was used to compare the one-year survival between the complete treatment group (who underwent surgery as local tumour control and chemotherapy) and the chemotherapy-only group. This study received ethical approval from the Health Research Ethical Committee of Dr. Hasan Sadikin General Hospital Bandung in May 2024 (approval number: DP.04.03/D.XIV.6.5/213/2024).

Results

Based on data collected from 2020 to 2023, applying exclusion criteria, there were 44 patients diagnosed with osteosarcoma, of which 27 were male and 17 were female (Table 1). Additionally, 14 patients were diagnosed with Ewing sarcoma, comprising 9 males and 5 females (Table 2).

Osteosarcoma was found in many age groups; 3 (6.8%) patients in the 0–5-year age group, 5 (11.4%) patients in the 6–10-year age group, and 9 (20.5%) patients in the 16–18-year age group (Table 1). The most prevalent age group of osteosarcomas was the 11–15-year age group, consisting of 27 (61.4%) patients (Table 1). In Ewing sarcoma, there are 4 (28.6%) patients in the 0–5-year age group, 4 (28.6%) patients in the 6–10-year age group, and 1 (7.1%) patient in the 16–18-year age group (Table 2). The most prevalent age group of Ewing sarcoma was the 11–15-year age group; it consists of 5 (33.7%) patients (Table 2).

Regarding laterality, osteosarcoma and Ewing sarcoma were more prevalent in unilateral involvement; 44 (100.0%) patients of osteosarcoma (Table 1) and 13 (92.9%) patients of Ewing sarcoma had unilateral involvement. Only 1 (7.1%) patient with Ewing sarcoma had bilateral involvement (Table 2).

The histopathological patterns in osteosarcoma patients varied. This study

Table 1 Characteristics of Osteosarcoma Patients

Characteristic	Category	n=44	%
Age (year)	0–5	3	6.8
	6–10	5	11.4
	11–15	27	61.4
	16–20	9	20.5
Gender	Male	27	61.4
	Female	17	38.6
Laterality	Unilateral	44	100
	Bilateral	0	0
Histopathological Type	Conventional Osteosarcoma	41	93.2
	Periosteal Osteosarcoma	2	4.6
	Telangiectatic Osteosarcoma	1	2.3
Primary Tumor Location	Femur	20	45.5
	Tibia	20	45.5
	Fibula	2	4.6
	Humery	2	4.6
Therapeutic Modality	Chemotherapy Only	27	61.4
	Complete Treatment (Chemotherapy + Surgery)	17	38.6
One Year Survival Outcome	Died	28	64.6
	Survived	16	36.4

Table 2 Characteristics of Ewing Sarcoma Patients

Characteristic	Category	n=14	%
Age (year)	0–5	4	28.6
	6–10	4	28.6
	11–15	5	33.7
	16–20	1	7.1
Gender	Male	9	64.3
	Female	5	35.7
Laterality	Unilateral	13	92.9
	Bilateral	1	7.1
Histopathological Type	Classical Ewing Sarcoma	14	100
Primary Tumor Location	Extremity	4	38.1
	Axial		
	Vertebrae	1	28.6
	Others	9	71.4
Therapeutic Modalities	Chemotherapy	8	57.1
	Complete Treatment (Chemotherapy + Surgery)	6	42.9
One Year Survival Outcome	Died	6	42.9
	Survived	8	57.1

found 41 (93.2%) patients with conventional osteosarcoma pattern, 2 (4.5%) patients with periosteal osteosarcoma pattern, and 1 (2.3%) patient with telangiectatic osteosarcoma pattern (Table 1). While in Ewing sarcoma, we only found classical Ewing sarcoma patterns in all (100%) patients (Table 2).

Location of the primary tumor in osteosarcoma was mostly found in the femur and tibia (n=20, 45.5%). The other locations found were in humerus of 2 (4.6%) patients and in the fibula of 2 (4.6%) patients (Table 1). In Ewing sarcoma, the most prevalent primary tumor location was in the axial part other than vertebrae, such as the head, neck, thorax, and abdomen (n=10, 71.4%). The other locations were in extremity of 4 (28.6%) patients and in vertebrae of 1 (7.1%) patient (Table 2).

Most of the osteosarcoma patients (61.4%) underwent chemotherapy alone. In contrast, among Ewing sarcoma patients, 8 individuals (57.1%) received chemotherapy, while the remaining 6 patients (42.9%) underwent chemotherapy combined with surgical intervention. There were various reasons why chemotherapy became the most chosen therapeutic modality, such as the parents rejecting the amputation or the patients coming in an unresectable condition.

The one-year survival rate for osteosarcoma patients was 36.4%, with 16 patients surviving, while 64.6% (28 patients) succumbed to the disease. Among Ewing sarcoma patients, 57.1% (8 patients) survived following therapy, whereas 42.9% (6 patients) died post-therapy.

Osteosarcoma patients who underwent chemotherapy combined with surgery demonstrated a higher one-year survival rate (n=12, 70.6%) compared to those who received chemotherapy alone (n=4, 14.8%) (Table 3). Data

Table 3 One-Year Survival Outcome in Osteosarcoma Patients by Treatment Type

	Survived (n)	Died (n)	p-value
Complete Therapy (Chemotherapy + Surgery)	12	5	0.000
Chemotherapy only	4	23	

Table 4 One-Year Survival Outcome in Ewing Sarcoma Patients by Treatment Type

	Survived (n)	Died (n)	p-value
Complete Therapy (Chemotherapy + Surgery)	6	0	0.000
Chemotherapy only	2	6	

on Ewing sarcoma patients also showed that those who underwent chemotherapy combined with surgery had a higher one-year survival rate (n=6, 100%) compared to chemotherapy alone (n=2, 25%) (Table 4).

Discussion

Osteosarcoma and Ewing Sarcoma are primary malignant bone tumors that predominantly affect pediatric populations. According to this research, the median age for osteosarcoma was 14.75 years, while the median age for Ewing Sarcoma was 9.25 years. Certain academic sources suggest that the peak age of onset of osteosarcoma falls within the range of 10 to 14 years, while Ewing sarcoma typically presents between 12 to 18 years.[6,8,9]

Gender disparities are seen in this study; 61.4% of individuals with osteosarcoma and 64.3% of individuals with Ewing Sarcoma were males. In line with previous studies, both osteosarcoma and Ewing Sarcoma demonstrate a higher incidence among male individuals compared to females. Williams LA et al. in their study found that the ratio of incidence in males and females varied depending on the timing of puberty. This is related to the peak of bone growth related to estrogen. Bone growth at puberty involves the growth hormone (GH)/insulin like growth factor 1 (IGF-1) axis. Research shows that estrogen increases GH secretion in both females and males, while testosterone affects GH secretion by converting it to estrogen through the aromatization process. Differences in the timing of puberty and estrogen levels in males and females affect bone growth and this explains the higher incidence of osteosarcoma and Ewing sarcoma in males during adolescence.[9–12]

Based on the results of this study, 93.2% of osteosarcoma in children at Dr. Hasan Sadikin General Hospital Bandung is conventional osteosarcoma. This is in line with some literature stating that conventional osteosarcoma is the classic type of osteosarcoma and is the most common histopathological pattern of osteosarcoma cases. Osteosarcoma is histologically classified by the World Health Organization (WHO) into central, intramodular, and surface. The central classification includes conventional osteosarcoma, telangiectatic osteosarcoma, small-cell osteosarcoma, and low-grade osteosarcoma. Conventional osteosarcoma is the most common type of osteosarcoma, representing 80% of all pediatric osteosarcoma cases. Conventional osteosarcoma is a high-grade tumor that originates from the intramedullary cavity.[13–15]

The most common primary tumor location of osteosarcoma in this study was the femur and tibia. This is consistent with the literature highlighting the prevalence of osteosarcoma in long bones. The femur, the longest and strongest bone in the human body, provides a conducive environment for the development of osteosarcoma due to its high vascularity and rapid bone growth during adolescence.[3]

On the contrary, Ewing sarcoma could happen in any bone or soft tissue; this uncertain predilection challenges the early detection of this sarcoma. In line with the literature, the primary tumor location of Ewing sarcoma in this study was found in various locations but mostly found in axial bones other than vertebrae. However, early detection and precise location of Ewing sarcoma can significantly impact treatment outcomes and improve survival rates for patients with this aggressive malignancy.[7]

One of the important prognostic factors in osteosarcoma and Ewing sarcoma is the laterality involvement of the disease. Unilateral involvement has a better prognosis. In this research, most of the patients, 44 (100%) osteosarcoma patients and 13 (92.9%) Ewing sarcoma patients, have unilateral involvement; this indicates that most patients had a better prognosis. This is evident in Ewing sarcoma patients, with 8 individuals (57.1%) surviving within one year. However, this is in contrast to osteosarcoma patients, the majority of whom (63.6%) died within one year after treatment. This may be due to the fact that many patients came in with complications and metastases, which delayed diagnosis and treatment. In addition, the fact that some patients' parents took

their patients to traditional treatment centers before coming to the hospital also delayed the diagnosis and treatment.

Patients with osteosarcoma who undergo chemotherapy and surgery have a higher one-year survival rate compared to those who receive chemotherapy alone. This is consistent with the research conducted by Liao et al., which indicates that patients treated with chemotherapy alone have significantly poorer outcomes and limited effectiveness in long-term results compared to those receiving a combination of surgical therapy and chemotherapy. Several studies also state that the administration of chemotherapy, particularly neoadjuvant therapy, increases the 5-year survival rate for osteosarcoma patients to 50-60%, compared to only 20% for surgery alone. Management of osteosarcoma with chemotherapy alone is insufficient to eradicate cancer cells in primary tumors or clinically detected metastases. One study conducted by Jaffe et al. showed that the use of chemotherapy alone as exclusive therapy for osteosarcoma has a low cure rate (10%), with nearly all patients experiencing disease recurrence and almost 50% developing pulmonary metastasis.[16,17]

This observation indicates a critical discussion on the impact of the limited use of treatment modalities on patient outcomes. Furthermore, the study results indicate that most patients with malignant bone tumors underwent only chemotherapy as a therapeutic modality. Chemotherapy can be a crucial component in the treatment of certain cancers, including bone tumors but, chemotherapy alone is insufficient to reliably destroy either the primary tumour or to eradicate clinically detectable metastases. Furthermore, the study results highlight the potential lack of utilization of other treatment modalities, such as surgery or radiation therapy, in the management of malignant bone tumors. This raises concerns about the comprehensiveness of care provided to these patients and whether a multidisciplinary approach involving different treatment modalities could lead to better outcomes.[3,7]

This study has several limitations that should be acknowledged. First, the sample size of 58 patients may not be representative of the entire population of childhood malignant bone tumor patients, potentially limiting the generalizability of the findings. Additionally, the study only included patients from a single institution, which may not capture the diversity of cases seen in different healthcare settings.

In conclusion, this study reveals critical characteristics of childhood malignant bone tumor patients at Dr. Hasan Sadikin General Hospital Bandung, highlighting the majority of osteosarcoma and Ewing sarcoma in male adolescents. Despite a higher incidence of unilateral tumors, the outcomes remain poor, with a significant number of patients experiencing death after 1 year of therapy. The findings highlight the urgent need for improved diagnostic and treatment strategies, as well as comprehensive care that includes various therapeutic modalities beyond chemotherapy. Addressing barriers to treatment access and promoting a multidisciplinary approach could enhance the prognosis and quality of life for these patients. Future research with a larger sample size and multi-center collaboration is recommended to further explore the factors influencing treatment decisions and patient outcomes and how high is the survival rate of patients with other multimodal therapies.

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Comparison of ABO Blood Group Antibody Titers in Elderly and Young Adult Patients

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Abstract

Immunosenescence in the elderly decreases their antibodies, which may lead to weaker degree of agglutination formation that potentially causes ABO discrepancies. This may lead to misinterpretation of blood group in this group. This study aimed to determine whether there are differences in antibody titer levels and degree of agglutination formed when examining ABO blood groups in the elderly as compared to young adults. This was a cross-sectional study employing analytical observational methods. Data were collected prospectively from the Blood Services Unit of Dr. Hasan Sadikin General Hospital Bandung, Indonesia, between May 2022 and July 2022. The subjects included were 42 elderly participants and 42 young adults, grouped accordingly. The ABO blood typing was performed using the slide method, while anti-A and anti-B titers were measured by serial two-fold dilution using the tube method. A significant difference in ABO blood group antibody titer levels was observed between elderly and young adult groups (median: 6 vs 64, $p<0.001$). Antibody titers were lower in the elderly across blood groups A (median: 8 vs 64, $p<0.001$), B (median: 8 vs 64, $p<0.001$), and O (median: 4 vs 64, $p<0.001$). The degree of agglutination was lower in the elderly (2+) compared to young adults (4+). On average, antibody titers in elderly individuals were approximately tenfold lower than those in young adults across all three blood groups. The reduced degree of agglutination further supports the diminished antibody response in the elderly group.

Keywords: ABO blood group, aged, antibodies, young adults

Introduction

Aging causes many changes in the physiological systems of the body. One of the important changes resulting from aging occurs in the body's immune system.[1] In the elderly it is known that their immune system decreases, which causes an increase in the elderly's vulnerability to infectious diseases, degenerative diseases, autoimmune diseases and malignancies. A decrease in the immune system in the elderly is called immunosenescence.[2] This condition causes a decrease in the innate and adaptive immune response. One of the most influential responses of aging to the immune system is a decreased ability to produce antibodies. The ability to differentiate, proliferate, and activate memory B cells decreases with increasing age, so the antibodies produced have a shorter duration of response than at a young age.[3]

In clinical practice, antibody activity is essential for ABO blood grouping, particularly in reverse typing, which detects plasma antibodies against A and B antigens. A reduction in antibody levels among the elderly may impair reverse typing, potentially leading to ABO discrepancies. The decrease in antibodies formed in the elderly can affect the reverse typing examination, causing a discrepancy between forward typing and reverse typing. This condition is known as ABO discrepancy, which can cause errors in the interpretation of blood groups which then lead to the administration of inappropriate blood groups which further leads to transfusion reactions.[4]

Transfusion is frequently required in elderly patients due to multiple comorbidities and higher surgical rates, with a reported

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transfusion prevalence of up to 62%. Some reasons for transfusion in elderly patients include the presence of comorbid conditions and the higher frequency of surgical procedures, making transfusions more common in older adults compared to younger individuals.[5] The high number of transfusion requests in the elderly shows the importance of appropriate procedures in carrying out transfusions to avoid transfusion reactions. ABO blood group examination using reverse typing is one of the precautions that can be taken to confirm blood group. Antibodies in the patient's plasma are reacted with a suspension of erythrocytes A, B, and O from healthy individuals whose blood group is known, then the agglutination formed is assessed. Weakened antibodies in the elderly can affect the degree of agglutination formed. Doubts in detecting agglutination can lead to misinterpretation of blood groups, resulting in discrepancies and can have life-threatening consequences for the patient.[6] Research shows that the incidence of ABO discrepancies due to decreased or weak antibodies reaches 9.2%, which often occurs among the elderly, newborns, and immunodeficiency patients.[7]

Weakening ABO antibody levels in the elderly can be identified by checking antibody titer levels and comparing them with healthy young adults. Several studies have mentioned how important it is to estimate ABO blood group antibody titer levels. ABO blood group titer levels can also provide information on the relative amount of antibodies present in the serum, and this information is very important in certain cases such as in operations requiring emergency blood transfusions, hemolytic disease in newborns, and in patients undergoing organ transplants.[8]

This study aims to determine whether there are differences in antibody titer levels and the degree of agglutination during ABO blood group testing between elderly individuals and healthy young adults at Dr. Hasan Sadikin General Hospital Bandung. The findings are expected to inform safer blood bank practices by enabling more accurate antibody detection and minimizing ABO discrepancies in elderly patients.

Methods

This study used an analytical observational approach with a cross-sectional design.. Data collection was conducted prospectively from May to July 2022 at the Blood Services Unit of Dr. Hasan Sadikin General Hospital Bandung, Indonesia. A total of 84 participants were included, consisting of 42 elderly individuals (aged ≥60 years) and 42 young adults (aged 26–45 years), each group meeting the age criteria established by the Ministry of Health of the Republic of Indonesia. Ethical approval was granted by the Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital (registration number: LB.02.01/X.6.5/305/2022.

The inclusion criteria were elderly outpatients treated at the geriatric clinic and young adult patients undergoing medical check-ups who had normal hematology results, including at least ten parameters: hemoglobin, hematocrit, leukocytes, erythrocytes, platelets, MCV, MCH, MCHC, RDW-SD, and RDW-CV.. The determination of the age limits of the two groups is based on the age criteria set by the Ministry of Health of the Republic of Indonesia. The exclusion criteria for this study were elderly outpatients aged ≥60 years with comorbid malignancies, autoimmune diseases, infections (seen from the patient's history of laboratory examination results in the last three months via the laboratory information system), and incomplete variable data.

ABO blood group typing was performed using forward and reverse typing via the slide method. The calculation of anti-A and anti-B titers was carried out using a serial two-fold dilution using 0.9% NaCl using the tube method. Then the examiner assesses the agglutination reaction in the tube that still provides the highest level of dilution and determines the patient's antibody titer level. Titer level checks were carried out on research samples with blood groups A, B, and O. Observations were carried out with the assistance of one observer other than the researcher to assess the degree of agglutination and antibody titer. A suitability test was carried out using the Cohen's Kappa test to assess the results of the examination between the researcher and the observer.

All data were recorded in Microsoft Excel. Subject characteristics were summarized using frequency distribution tables. Differences in antibody titer levels between the elderly and young adult groups were analyzed using the Mann–Whitney test. Statistical analysis was conducted using SPSS version 25.

Results

Table 1 shows that the elderly group was predominantly male (76.2%), while the young

Table 1 Characteristics of Research Subjects

Variables	Elderly (n=42) n (%)	Young Adults (n=42) n (%)	p-value
Sex			<0.001[a]
Male	32 (76.2)	12 (28.6)	
Female	10 (23.8)	30 (71.4)	
Age (year)			
Mean ± SD	67.5±5.1	34.1±4.8	<0.001[b]
Blood Group			1.000[c]
A	11 (26.2)	11 (26.2)	
B	13 (31.0)	15 (35.7)	
O	18 (42.9)	16 (38.1)	
Degree of Agglutination in Reverse Typing			<0.001[c]
1+	2 (4.7)	0 (0.0)	
2+	28 (66.7)	0 (0.0)	
3+	12 (28.6)	8 (19.0)	
4+	0 (0.0)	34 (81.0)	

[a]Test for different gender proportions using the Chi-Square test; [b]Test for differences in age means using the Mann-Whitney test;[c]Test for different blood group proportions and degrees of agglutination using the Kolmogorov-Smirnov test

Table 2 Comparison of ABO Blood Group Antibody Titers between Elderly and Young Adults

ABO Antibody Titer Levels	Elderly n=42	Young Adults n=42	p-value
ABO Blood Group			
Median (Min-Max)	6 (2–8)	64 (32–128)	<0.001[a]*
<32	42 (100)	0 (0)	<0.001[b]*
≥32	0 (0)	42 (100)	
A Blood Group (n=11)			
Median (Min-Max)	8 (2–8)	64 (32 – 128)	<0.001[a]*
<32	13 (100)	0 (0)	<0.001[b]*
≥32	0 (0)	16 (100)	
B Blood Group (n=13) (n=16)			
Median (Min-Max)	8 (4–8)	64 (32–128)	<0.001[a]*
<32	13 (100)	0 (0)	<0.001[b]*
≥32	0 (0)	16 (100)	
O Blood Group (n=18, 15)			
Median (Min-Max)	4 (2–8)	64 (32–64)	<0.001[a]*
<32	18 (100)	0 (0)	<0.001[b]*
≥32	0 (0)	15 (100)	

[a]Mann Whitney, [b]Chi Square

adult group was predominantly female (71.4%). The average age was 67.5 years in the elderly group and 34.1 years in the young adult group. The proportion of blood groups A, B, and O in the two groups showed no significant difference. However, the results obtained from this study show that the blood group O dominated the blood group of the research subjects.

Based on the degree of agglutination, the elderly group is dominated by 2+ with a few showing a degree of 3+. However, in young adults, the degree of agglutination is dominated by agglutination degree 4+. The results of the average ABO blood group antibody titer in elderly patients and young adults showed significant results, that the average antibody titer in the elderly was 10 times lower than in young adults. Between reader 1 and reader 2, a conformity test was carried out between the antibody titer readings using the Kappa test, and a value of 0.817 was obtained, which means a strong agreement with a p-value <0.001.

The results of the analysis in Table 2 show that there are differences in ABO blood group antibody titer levels between the elderly and healthy young adults at Dr. Hasan Sadikin General Hospital Bandung (median: 6 vs 64, p<0.001). Antibody titer levels were lower in the elderly compared to young adults in both blood groups A (median: 8 vs 64, p<0.001), B (median: 8 vs 64, p<0.001), and O (median: 4 vs 64, p< 0.001).

Discussion

In this study, the elderly group was predominantly male, which contrasts with findings from Madyaningrum et al.who reported that 51.6% of elderly outpatient visits were female.s. In this research, several factors influence the number of visits to elderly hospital clinics in Indonesia, including the presence or absence of health insurance, where at that time the BPJS national health insurance had only been running for one year. Other factors include economic status, religion, level of education, level of awareness of personal health conditions, and comorbidities suffered by the patient.[9] Further research is needed to determine the number of patient visits and the factors that influence visits at the elderly clinic at Dr. Hasan Sadikin General Hospital Bandung.

In this study, differences were found in the degree of agglutination formed in the reverse typing examination. The degree of agglutination in the elderly is lower with the average agglutination being 2+, which can be seen from the formation of several small clumps with clear areas around them. In contrast, young adults have an agglutination average of 4+, which appears as large lumps. Erythrocyte agglutination can take place in two stages. The first stage is that the antibodies bind to the surface of the erythrocytes. This is then continued with the second stage, in which the antibodies interact with erythrocytes so that adjacent cells agglutinate. The first stage of agglutination is influenced by temperature, medium pH, antibody affinity constant, incubation time, ionic strength in the medium, and antibody-antigen ratio. The second stage of agglutination is influenced by the distance between cells, the charge of the molecules in the suspension, membrane deformity, membrane surface molecules, and molecular structure.[6]

In this studyslide method, the degree of agglutination is influenced by the antibody-antigen ratio, especially since immunosenescence occurs in the elderly. The ratio of antigen and antibody is very important in determining the strength of the reaction. The more antibodies that bind to the antigen on the surface of the erythrocytes, the stronger the reaction will be. Weak or missing antibodies found in old age (elderly) can cause the agglutination that forms to become weak.[6] In accordance with the results of this study, the antibody titer in elderly patients is 10 times lower than in young adults, so the degree of agglutination formed is also lower.

This research is in accordance with the research by Saidin, et al. which examined ABO antibody levels from 311 donor samples. It was found that the levels of anti-A and anti-B titers were low in people aged over 50 years, with an average titer < 1:64. The highest titer levels were found in the age range 18-29 years with an average titer ≥1:64.[10] The average age of young adults in this study was 34 years with an average titer of 1:60. Meanwhile, elderly people under 65 years of age have an average titer of 1:6. This occurs because, in the humoral immune system, the production of B lymphocytes gradually decreases due to aging so the ability to produce antibodies also decreases. The ability to differentiate, proliferate, activation of memory B cells decreases and when antibodies are produced, the duration of presence of the antibody response in the elderly is shorter than in the young.[11]

Beyond aging, anti-ABO levels may also be influenced by ethnicity, diet, lifestyle, and environmental exposure. The high titer of ABO antibodies in Asian and African populations has

been suggested to be caused by mosquito bites and parasitic intestinal infections. Many bacteria, viruses, and parasites have surface antigens that resemble ABO blood group antigens. Chronic exposure to these pathogens can lead to the production of cross-reactive antibodies, boosting ABO antibody levels. Asian and African countries have large forest areas, increasing the risk of mosquito bites. The study by Kannan et al., found a significant relationship between types of diet and high antibody titers. Donors with a vegetarian diet have a higher chance of obtaining high titer antibodies (≥1:64) compared to those with a mixed diet (<1:64).[12] However, food consumption factors were not examined in this study.

This study has several limitations. Factors that could influence ABO antibody titers—such as vaccination history, infection status, pregnancy history in female subjects, and methodological limitations of the antibody titer assay—were not considered. Furthermore, the use of the conventional tube method, which is inherently subjective, may have introduced variability in interpretation across examiners.[13] Inconclusion, the ABO antibody titer in the elderly is 10 times lower and the degree of agglutination formed is also lower compared to young adults. Although this does not cause differences in blood group interpretation, it is still important for elderly patients to have their ABO blood group checked using reverse typing to prevent misinterpretation due to low ABO antibody titers.

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Correlation of Nutritional Status, HbA1c, and Duration of Diabetes Mellitus with Amputation Incidence in Patients with Diabetic Foot Ulcers

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Abstract

Diabetic foot ulcer (DFU) is a severe complication of diabetes that significantly impairs quality of life and may often lead to amputation, particularly when infections become extensive. This study investigated the correlation of nutritional status, HbA1c levels, and duration of diabetes exposure with the incidence of amputation in DFU patients. Using an observational analytic study with a retrospective cohort design, data from 47 DFU patients treated at the Vascular and Endovascular Surgery clinic and Emergency Room of a hospital from 2019-2024 were analyzed. The majority of subjects were men aged 40-60 years (59.57%). Among the amputee group, 55.31% experienced neuropathy, primarily classified as Wagner's degree 4. Patients requiring amputation had significantly higher levels of urea, creatinine, and leukocytes compared to those who did not. A significant correlation was observed between the incidence of amputation and nutritional status—specifically, serum albumin (r = -0.616) and Body Mass Index (BMI) (r = 0.823)—as well as HbA1c levels (r=0.806) and duration of diabetes exposure (r=0.445) ($p<0.05$). However, the Subjective Global Assessment (SGA) did not show a significant relationship with amputation incidence. The findings of this study suggest that nutritional status, HbA1c levels, and duration of diabetes exposure are significantly correlated with the likelihood of amputation in patients with diabetic foot ulcers (DFU).

Keywords: Amputation, diabetes mellitus, diabetic foot ulcer, HbA1c, nutritional status

Introduction

Diabetic Foot Ulcer (DFU) is a severe complication of Diabetes Mellitus (DM), occurring when a diabetic patient develops a wound. It results from the loss of sensory and motor neuropathy, peripheral vascular disorders, and prolonged infections.[1] Globally, DM affects 9.3% of the population and is among the top 10 deadliest diseases. DFU prevalence is highest in the U.S. (13%), followed by Africa (7.2%), Asia (5.5%), and Europe (5.1%).[2] In Indonesia, there is no specific data on DFU prevalence, but DM cases are expected to increase by 8.2 million by 2020.[3] Amputation is a significant risk for DFU patients, with one limb lost every 30 seconds worldwide, and high mortality rates following amputation (14.3% within a year and 37% within three years).[4]

Around 25% of DM patients may develop DFU, and 16% of those may require amputation if not treated properly.[5] Poor nutritional status and high HbA1c levels (>10%) are major factors affecting wound healing and increasing

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amputation risk.[6] Diabetic foot ulcers are one of the common complications in diabetes patients and are a complication of diabetes mellitus that can lead to increased hospital care costs.[7] The high prevalence of DFU among DM patients indicates inadequate prevention and treatment. This is the first study to analyze the significance of nutritional status, HbA1c levels, and duration of DM exposure with risk of amputation in West Java. This study aims to analyze the correlation between nutritional status, HbA1c levels, and the duration of DM exposure with the risk of amputation in patients at Dr. Hasan Sadikin Hospital Bandung, Indonesia.

Methods

This study is an observational analytic study with a retrospective cohort design. All samples that meet the inclusion criteria and are not exposed to the exclusion criteria are included as research subjects. The subjects of this study are patients with diabetic foot ulcers (DFU) who received treatment at the Vascular and Endovascular Surgery Clinic and the Emergency Department of Dr. Hasan Sadikin General Hospital from January 2019 to June 2024. The subjects involved in this study are those who meet the inclusion criteria and are not affected by the exclusion criteria.

Patients included in this study are patients diagnosed with diabetic foot ulcers (DFU), who seek treatment at the Vascular Surgery Clinic and Emergency Department of RSHS, aged above 18 years, and diagnosed with Type 2 Diabetes Mellitus. Patients who are pregnant, patients with incomplete medical records, patients with a history of prior amputation are excluded in this study. Data on the characteristics of the subjects are collected from patient medical records. The sampling process is carried out using the consecutive sampling method.

This study was approved by the Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital, Bandung, under the reference number DP.04.03/D.XIV.6.5/38/2024. The study prioritizes key ethical aspects, particularly the confidentiality of patient medical records.

Data normality was assessed using the Shapiro–Wilk test. Statistical tests were then applied to evaluate relationships between numerical and categorical variables. Correlation strength is evaluated using Guilford's criteria. Correlation direction and p-values determine significance, with $p \leq 0.05$ indicating statistical significance. Data are analyzed using SPSS version 24.0 for Windows.

Result

This study is a retrospective cohort study conducted at Dr. Hasan Sadikin General Hospital Bandung, using data from 2019 to 2024. It includes 47 patients who met the inclusion criteria, with no exclusions. Data collected and analyzed include age, gender, neuropathy, Wagner grade, levels of urea, creatinine, leukocytes, and blood. Nutritional status variables assessed include albumin levels, BMI, and SGA scores. The study also evaluates HbA1c levels, duration of diabetes exposure, and amputation occurrences. Table 1 shows that most patients are 40–60 years old and predominantly male. Neuropathy is common, especially in those needing amputation (55.3%) compared to those who don't (14.9%). Most amputations are Wagner grade 4, and patients requiring amputation have higher levels of urea, creatinine, and leukocytes. This study also divides amputations into major and minor categories, with a higher prevalence of major amputations among working-age patients. Major amputation patients have significantly higher urea, creatinine, and leukocyte levels.

Table 1 also indicates that amputated patients have lower serum albumin (1.40 g/dL) compared to non-amputated ones (1.99 g/dL), with major amputation patients having the lowest levels (1.35 g/dL). BMI is higher in the amputated group, with the highest average BMI found in major amputation patients. Most subjects are classified as SGA B, with a consistent distribution of SGA type C across amputation groups.

In this study, nutritional status variables showed a normal distribution and were analyzed using Spearman's correlation. Significant correlations were found: serum albumin had a strong negative correlation with amputation risk ($r=-0.6$, $p<0.001$), while BMI had a very strong positive correlation with amputation risk ($r= 0.8$, $p<0.001$). Chi-Square tests showed that SGA was not significantly related to amputation ($p>0.05$).

Table 3 shows that HbA1C levels are significantly higher in the amputation group compared to the non-amputation group. Within the amputation group, those requiring major amputation have higher average HbA1C levels than those requiring minor amputation. The highest HbA1C level observed was 9.80% in the major amputation group.

The study found that HbA1c levels are normally distributed, with Spearman correlation

Table 1 Subject Characteristics

Variable	Total (n=47)	Not amputated	Amputated	Minor Amputation	Major Amputation
Age (year)					
<40	1 (2.1)	0	1 (2.1)	1 (2.1)	0
40–60	8 (59.6)	12 (25.5)	16 (34.0)	5 (10.6)	11 (23.4)
>60	18 (38.3)	7 (14.9)	11 (23.4)	3 (6.4)	8 (17.0)
Sex					
Male	24 (51.1)	12 (25.5)	12 (25.5)	3 (6.4)	9 (19.1)
Female	23 (48.9)	7 (14.9)	16 (34.0)	6 (12.8)	10 (21.3)
Neuropathy					
Yes	33 (70.2)	7 (14.9)	26 (55.3)	8 (17.0)	18 (38.3)
No	14 (29.8)	12 (25.5)	2 (4.3)	1 (2.1)	1 (2.1)
Wagner Classification					
Grade 1	1 (2.1)	1 (2.1)	0	0	0
Grade 2	4 (8.5)	4 (8.5)	0	0	0
Grade 3	13 (27.7)	10 (21.3)	3 (6.4)	1 (2.1)	2 (4.3)
Grade 4	26 (55.3)	4 (8.5)	22 (46.8)	7 (14.9)	15 (31.9)
Grade 5	3 (6.4)	0	3 (6.4)	1 (2.1)	2 (4.3)
Ureum (mg/dL)					
Mean ± SD	80.3±43.9	40.0±21.3	107.5±32.6	99.8±24.3	111.2±35.9
Median (Range)	80.4 (11.2–177.4)	38.0 (11.2–80.4)	96.3 (64.7–177.4)	98.4 (68.0–133.4)	95.0 (64.7–177.4)
Creatinine (mg/dL)					
Mean ± SD	1.4 ± 0.5	0.9 ± 0.3	1.8 ± 0.3	1.7 ± 0.2	1.8 ± 0.3
Median (Range)	1.5 (0.5–2.4)	0.9 (0.5–1.3)	1.8 (1.3–2.4)	1.8 (1.3–2.1)	1.8 (1.4–2.4)
Leucocyte (mm^3)					
Mean ± SD	23,909.4 ± 10,526.0	14,243.7 ± 5,812.2	30,468.2 ± 7,475.9	22,725.6 ± 2,180.3	34,135.8 ± 6,127.6
Median (Range)	23,980 (7,320–47,050)	13,160 (7,320–33,800)	28,655 (19,300–47,050)	23,340 (19,300–25,220)	34,780 (25,320–47,050)
Serum albumin (g/dL)					
Mean ± SD	1.6 ± 0.5	2.0 ± 0.5	1.4 ± 0.3	1.5 ± 0.3	1.4 ± 0.3
Median (Range)	1.6 (0.9–3.0)	2.0 (1.3–3.0)	1.4 (0.9–1.9)	1.4 (1.2–1.9)	1.3 (0.9–1.9)
BMI (kg/m^2)					
Mean ± SD	24.9 ± 4.4	20.8 ± 2.1	27.7 ± 3.1	28.3 ± 3.3	27.4 ± 3.1
Median (Range)	25.4 (18.2–35.2)	19.8 (18.2–24.7)	26.8 (22.8–35.2)	28.7 (23.5–33.6)	26.7 (22.8–35.2)
Subjective Global Assessment (SGA)					
A (Well Nourished)	10 (21.3)	4 (8.5)	6 (12.8)	2 (4.3)	4 (8.5)
B (Moderate Malnourished)	34 (72.3)	14 (29.8)	20 (42.6)	6 (12.8)	14 (29.8)
C (Severe Malnourished)	3 (6.4)	1 (2.1)	2 (4.3)	1 (2.1)	1 (2.1)

Table 2 Correlation Between Nutritional Status and Amputation Incidence

Variable	Total (n=47)	Not amputated	Amputated	p-value	r value
Serum albumin (g/dL)				<0.001	–0.6
Mean ± SD	1.6 ± 0.5	2.0 ± 0.5	1.4 ± 0.3		
Median (Range)	1.6 (0.9–3.0)	2.0 (1.3–3.0)	1.4 (0.9–1.9)		
BMI (kg/m^2)				< 0.001	0.8
Mean ± SD	24.9 ± 4.4	20.8 ± 2.1	27.7 ± 3.1		
Median (Range)	25.4 (18.2–35.2)	19.8 (18.2–24.7)	26.8 (22.8–35.2)		
SGA				0.071	
A (Well nourished)	10 (21.3)	4 (8.5)	6 (12.8)		
B (Moderate malnourished)	34 (72.3)	14 (29.8)	20 (42.6)		-
C (Severe malnourished)	3 (6.4)	1 (2.1)	2 (4.3)		

Table 3 HbA1c Status Characteristics by Amputation Status

Variable	Total	Not amputated	Amputated	Minor Amputation	Major Amputation
HbA1c (%)					
Mean	7.7	6.4	8.6	8.0	8.8
Standard deviation	1.4	0.5	0.3	0.6	0.7

analysis showing a very strong relationship (r=0.8) between HbA1c levels and amputation incidence (p<0.001). Higher HbA1c levels correlate with a greater likelihood of amputation (Table 4).

Table 5 shows a nearly 3-year difference in diabetes duration between amputated and non-amputated groups. However, minor amputations have a higher average duration of diabetes exposure compared to major amputations. The maximum duration observed is 12 years in the major amputation group.

The analysis of diabetes duration, which is normally distributed, used Spearman's correlation test. Results indicate a significant correlation with a p-value less than 0.05 (p= 0.001) and a moderate correlation coefficient (r=0.4). This suggests that a longer duration of diabetes exposure increases the likelihood of amputation.

Discussion

Diabetes Mellitus (DM) is a chronic condition with rising prevalence, leading to significant morbidity and mortality. Complications like circulatory disorders and neuropathy can cause chronic wounds and infections, potentially resulting in limb amputation. Amputation impacts patients' quality of life and adds economic and psychological burdens. This study

Table 4 Correlation of HbA1c Levels With Amputation Incidence

	Total	Not Amputated	Not amputated	p-value	r value
HbA1c (%)					
Mean ± SD	7.7±1.4	6.4±0.5	8.6±0.3	<0.001	0.8

Table 5 Characteristics of Duration of DM Exposure

Variable	Total	Not amputated	Amputated	Minor Amputation	Major Amputation
Duration of DM (years)					
Mean	6.0	4.5	7.1	7.3	6.9
Standard deviation	2.9	2.6	2.7	2.7	2.8

Table 6 Correlation of DM Duration with Amputation Incidence

Variable	Total (N)	Not amputated	Amputated	p-value	r value
Duration of DM (years)					
Mean ± SD	6.0±2.9	4.5±2.6	7.1±2.7	0.001	0.4

identifies factors influencing amputation risk in DM patients to aid in prevention and early intervention. Data is categorized into major and minor amputations to enhance understanding and improve preventive measures.

The majority of DM patients in this study were within the working-age group (40–60 years), accounting for 59.57% of cases. This finding aligns with research by Abbott et al., which noted that DM tends to manifest at a younger age in Asian populations compared to African and Caucasian groups.[8] Diabetic neuropathy is three times more common in patients requiring amputation compared to those who do not. Neuropathy leads to poor circulation in the feet, causing loss of pain sensation and increasing the risk of infections and ulcers. Perveen's research indicates 50% of DM patients experience neuropathy.[9]

DM can damage small blood vessels in the kidneys, impairing their function and leading to elevated blood urea and creatinine levels, which are markers of kidney function. Higher levels are observed in major amputation cases compared to minor ones, and Gazzaruso et al. research supports these findings.[10] Elevated leukocyte levels, indicating inflammation or infection, are also twice as high in patients needing amputation. Jiang et al. found a link between increased leukocytes and a higher risk of major amputation.[11]

The leukocyte data aligns with findings showing that most amputated subjects have a Wagner grade of 4, indicating localized gangrene due to infection and circulation issues. This mirrors Wang et al.[12] research, which found most surgical patients had Wagner ≥ 3.

Significant differences were found in average serum albumin levels between amputated and non-amputated patients. Non-amputated patients had higher average serum albumin (1.99 g/dL) compared to amputated patients (1.40 g/dL). This suggests that higher serum albumin levels are associated with a lower risk of amputation, as serum albumin is a key indicator of nutritional and overall health status. These findings are consistent with Gulcu et al. in Turkey, which identified a significant relationship between albumin levels and amputation risk in diabetic patients. Lower albumin levels correlate with higher amputation risks ($p < 0.05$).[12]

Low serum albumin may indicate protein metabolism disorders, liver dysfunction, or malnutrition, leading to decreased tissue strength, infection resistance, and wound healing, thereby increasing amputation risk. This study observed that patients requiring major amputations had lower average serum albumin levels than those needing minor amputations. The relationship between serum albumin and amputation risk can be attributed to its role in transporting essential substances and reflecting nutritional and liver health. Low albumin levels may worsen vascular and neuropathic complications in diabetes, increasing amputation risk.[13] Chronic inflammation and underlying health conditions can also elevate amputation risk. Chronic inflammation associated with type 2 diabetes can cause vascular damage, immune system disruption, and delayed wound healing, exacerbating foot ulcers and amputation risk.[14] Therefore, serum albumin is a crucial indicator of general health and complication risk.

There were significant differences in BMI between amputated and non-amputated patients. Non-amputated patients had a lower

average BMI (20.77 kg/m^2), while amputated patients had a higher average BMI (27.69 kg/m^2). Surprisingly, minor amputation patients had a higher average BMI than major amputation patients, despite literature suggesting higher BMI is linked to increased amputation risk. This aligns with Costa et al.[15] in Brazil, where a significant relationship between BMI and amputation risk was found. Higher BMI is associated with increased amputation risk due to factors like obesity-related vascular problems and worsened neuropathy.[16] Obesity can exacerbate diabetic complications, affecting wound healing and increasing ulcer risk.[16] Managing weight and preventing obesity are crucial for reducing amputation risk, with interventions focusing on weight loss, diet, and physical activity.

The study found that most patients with diabetic complications had moderate malnutrition (72.3%) based on SGA. Although no significant difference in amputation rates was observed among SGA groups (p=0.071), malnutrition is linked to increased amputation risk. Zhu et al.[17] in China also found malnutrition increases amputation risk in diabetic patients (p<0.05). Malnutrition affects amputation risk through impaired immune function, delayed wound healing, and worsened diabetic complications. Proper nutritional management is vital for reducing amputation risk and improving clinical outcomes.[18]

Significant differences were observed in average HbA1c levels between amputated (8.6%) and non-amputated patients (6.4%). Higher HbA1c levels were noted in major amputations (8.8%) compared to minor ones (8.0%). This highlights the importance of strict blood glucose control in preventing serious complications like amputation. These findings are consistent with Shatnawi et al.[19], where higher HbA1c levels were associated with increased amputation risk (p<0.001). Poor glucose control leads to vascular and neuropathic complications, increasing amputation risk.[19]

High HbA1c indicates chronic hyperglycemia causing vascular and peripheral nerve damage, leading to difficult-to-heal wounds and increased amputation risk. Poor glucose control also exacerbates peripheral vascular disease and reduces oxygen and nutrient supply to the extremities.[20]

The study found that most patients had DM for over 5 years, with amputated patients having a longer exposure (7.07 years) compared to non-amputated ones (4.47 years). Significant differences in amputation rates were noted with different DM durations (p = 0.001), showing that longer DM duration affects amputation risk.[18] This is consistent with Nouira et al.[21] and Shatnawi et al.[19] where longer DM duration was significantly associated with higher amputation rates. However, the study's findings showed similar or even longer DM durations in minor amputations, possibly due to limited data.[21]

Long-term DM increases risk through accumulated damage from chronic hyperglycemia, affecting small and large vessels and peripheral nerves. Complications like microangiopathy and neuropathy worsen circulation and wound healing, raising amputation risk.[22] Proper glycemic management is crucial, regardless of DM duration.

The study's limitations include potential selection bias from consecutive sampling, medical records, and lack of differentiation between ischemic and neuropathic causes of complications. Additionally, the use of existing medical data may introduce unrecognized confounding biases affecting the interpretation of amputation risk factors. The secondary data also limits the ability to explore variables that were not initially intended for analysis in this research, potentially reducing the depth and accuracy of findings.

This study concludes that diabetic patients, particularly those in the productive age range (40–60 years), face significant risks of amputation due to complications such as diabetic neuropathy, poor renal function, and malnutrition. Higher levels of HbA1c and longer durations of diabetes are strongly associated with increased amputation risks, underscoring the critical importance of effective glycemic control and early management of diabetes. Additionally, while higher BMI is observed in amputated patients, it does not directly correlate with amputation severity. Overall, the findings highlight the need for proactive intervention and comprehensive care to prevent severe diabetic complications and reduce the risk of amputations.

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Factors Associated with Soil-Transmitted Helminths Infections in Children Aged 24–59 Months in Bandung District, Indonesia

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Abstract

Soil-transmitted helminth (STH) infections remain a major public health concern in developing countries, particularly among lower- to middle-income populations with poor sanitation, limited access to healthcare, and inadequate clean water. Preschool-aged children are especially vulnerable due to their developing immune systems and increased nutritional needs. This study aimed to identify factors associated with STH infections among children aged 24–59 months in Bandung District, Indonesia. A case-control design was employed using secondary data from helminthiasis surveillance conducted between October 2019 and January 2023, involving a total of 261 children. The Kato-Katz technique was used to examine stool samples for the detection of STH species. Among them, 30 (11.5%) were infected with soil-transmitted helminths (STH), comprising ascariasis (11.1%), trichuriasis (0.4%), and hookworm infection (0.4%). Bivariate chi-square analysis revealed significant associations with fathers' education level (p=0.0003), BPJS-Healthcare participation (p=0.015), water source (p=0.015), distance from the water source to the latrine (p=0.003), and nail hygiene (p=0.018). Multivariate logistic regression confirmed that distance between water source and pit latrine (OR= 0.265; 95% CI: 0.76–0.92), nail hygiene (OR = 0.318; 95% CI: 0.13–0.76), and participation in BPJS-healthcare program (OR=0.364; 95% CI: 0.15–0.91) were key determinants of STH infection. These findings highlight the importance of addressing environmental and behavioral factors through public health interventions, including improving access to clean water, promoting personal hygiene, and enhancing parental, particularly paternal education, as essential strategies for reducing the risk of STH in children.

Keywords: Children, helminths, helminthiasis, risk factors

Introduction

Intestinal parasitic infections are a prevalent global health issue, specifically in developing nations. The primary contributors to these infections are helminths, known as soil-transmitted helminths (STH), which are spread through contact with contaminated soil.[1] In addition, STH is one of the 20 neglected tropical diseases, primarily prevalent in tropical regions and often ignored in global health agendas.[2] The main STH species that infect humans include roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), and hookworm species (*Necator americanus* and *Ancylostoma duodenale*).[3]

According to the World Health Organization (WHO), approximately 1.5 billion people, or 24% of the world's population, are infected with STH, and over 260 million preschool-age children are at risk of infections by these parasites.[4] The high prevalence of STH infections has been reported to be associated with low education levels, poor socioeconomic status, inadequate sanitation, limited medical services, and restricted access to clean water. Warm, humid climates also promote helminths growth, further contributing to the spread of infections through contaminated soil. These infections are also among the leading causes of morbidity and mortality,

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disproportionately impacting populations in lower to middle-income settings.[2,5]

WHO identifies preschool-aged children (2–5 years of age) as a high-risk group for soil-transmitted helminth (STH) infections and schistosomiasis, emphasizing their vulnerability to adverse effects due to their heightened need for micronutrients.[4] In addition to their susceptibility to STH, children in this age group are particularly vulnerable to stunting and malnutrition, which further exacerbates their risk.[6,7] The prevalence of STH infections in stunted children, ranges from 12.5% to 56.5%, primarily due to immune disturbances, inflammation, and gut microbiota imbalances, thereby increasing their susceptibility.[8] STH infections can cause decreased appetite, nutrient absorption issues, iron deficiency, and gastrointestinal problems, leading to malnutrition, stunting, cognitive impairment, and long-term health risks.[9] Although many studies have explored biological and behavioral factors contributing to STH infections, research focusing on sociodemographic factors remains limited. Therefore, this study aims to provide a comprehensive overview of the factors associated with soil-transmitted helminth (STH) infections among children aged 24–59 months in Bandung Regency, as addressing these factors is crucial for reducing STH infections and consequently lowering childhood morbidity and mortality.[10] By identifying the factors associated with STH infection, this study aims to guide targeted public health strategies to reduce STH infections and improve health outcomes in children.

Methods

This study was a retrospective case-control design using secondary data from helminthiasis surveillance conducted between October 2019 and January 2023.. Medical records were collected following ethical exemption approval from the Health Research Ethics Committee of Universitas Padjadjaran (No. 98/UN6.KEP/EC/2024). The minimum sample size required for a 95% confidence interval (CI) was 27 participants, which was calculated by the following formula:

$$n1 = n2 = \left(\frac{Z\alpha\sqrt{2PQ} + Z\beta\sqrt{P_1Q_1 + P_2Q_2}}{P_1 - P_2} \right)^2$$

$$n1 = n2 = \left(\frac{1.96\sqrt{2 \text{ x } 0.2 \text{ x } 0.8} + 0.84\sqrt{0.35 \text{ x } 0.65 + 0.05 \text{ x } 0.95}}{0.35 - 0.05} \right)^2$$

$$n1 = n2 \approx 27$$

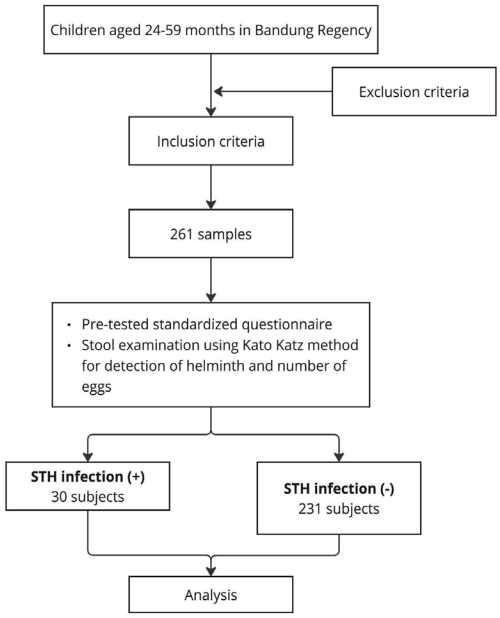

Figure 1 Flowchart of the Participant Selection Process for the Study

This descriptive-analytical study applied a case-control approach to investigate risk factors associated with helminthiasis among children aged 24 to 59 months in Bandung Regency. A multistage random sampling method was used to obtain a representative sample. Following the selection of 13 districts, 39 villages, and 29 primary health care centers, a simple random sampling technique was used to select children with helminthiasis. The inclusion criteria included children aged 24 to 59 months who resided in Bandung Regency during the study period, while exclusion criteria consisted of incomplete data, recent anthelmintic drug consumption, and congenital disorders or specific syndromes.

Stool analyses were carried out by a certified laboratory at Dr. Hasan Sadikin Central General Hospital. The Kato-Katz technique was used to detect species of STH, such as *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms. Data collected included infection type, age, nutritional status, and related factors. Statistical analysis was conducted using Microsoft Excel and SPSS version 27. Bivariate analysis using chi-square tests was applied to determine the association between risk factors and helminthiasis. Multivariate logistic regression was used to evaluate the simultaneous influence of multiple

independent variables. The odds ratio (OR) for the binary outcome variable was evaluated through the univariate analysis. Variables with a p-value < 0.25 were subsequently examined using multivariate analysis to account for potential confounders. Consequently, the final model was reported using the OR with a 95% CI. Statistical test results were considered to exhibit a significant causal relationship when p-value <0.05.

Results

Data were collected from October 2019 to January 2023, with 261 children being included in the study. This consisted of 30 children with STH infections (11.5%) and 231 children without STH infections (88.5%). The majority of children with STH infections suffered from ascariasis (11.1%), followed by trichuriasis (0.4%) and hookworm infections (0.38%). In addition, single infections (11.1%) was the most common, followed by a small percentage experiencing dual infections of ascariasis with hookworm (0.4%).

According to the descriptive analysis, it was observed that 150 children (57.5%) were boys, and 111 (42.5%) were girls. Overall, the average age of the participants was 2.83 years old. The nutritional status characteristics of children were grouped into nutritional status based on height-for-age and weight-for-age. In addition, it was found that 18.8% and 24.1% of children suffered from underweight and severely underweight conditions, respectively. This study also showed that 21.5% and 34.5% of children suffered from stunting and severe stunting conditions, respectively. Additional detailed information regarding the characteristics of these children was presented in Table 1.

Variables associated with STH infections were analyzed using bivariate chi-square and multivariate logistic regression. In addition, factors were considered significant when their p-value was <0.05. Based on bivariate chi-square analysis, having a father with a secondary school education (OR= 0.159; 95% CI: 0.21-1.29; p=0.0003), father with a high school education (OR=0.226; 95% CI: 0.87-0.59; p=0.0003), father with a college education (OR=0.735; 95% CI: 0.14-3.98; p=0.0003), participation in BPJS-healthcare program (OR=0.218; 95% CI: 0.06-0.74; p=0.015), water pump as the source of water (OR=0.527; 95% CI: 0.15-1.82; p=0.014), distance between the water source and the pit latrine (OR=0.268; 95% CI: 0.11-0.63; p=0.002), and nail hygiene (OR=0.322; 95% CI: 0.13–0.77; p=0.018) were identified as 7 significant factors associated with STH infections incidence. The association between factors of STH infections and the incidence of STH infections was highlighted in Table 2.

Table 3 shows the results of the multiple logistic regression analysis, which was conducted in three sequential steps. This

Table 1 Characteristics of Children Enrolled in the Study (n=261)

Variable	n (%)
Sex	
Male	150 (57.5%)
Female	111 (42.5%)
Age	
2 years	121 (46.4%)
3 years	62 (23.7%)
4 years	78 (29.9%)
Father's education	
Primary	50 (19.2%)
Secondary	103 (39.5%)
High school	99 (37.9%)
College	9 (3.4%)
Mother's education	
Primary	54 (20.7%)
Secondary	114 (43.7%)
High school	83 (31.8%)
College	10 (3.8%)
Monthly income (IDR)	
500.000–2 million	119 (45.6%)
2–3.5 million	117 (44.8%)
3.5–5 million	12 (4.6%)
>5 million	13 (5.0%)
STH infections	
Ascariasis	29 (11.1%)
Trichuriasis	1 (0.4%)
Hookworm	1 (0.4%)
Infections types	
Single infections	29 (11.1%)
Dual infections	1 (0.4%)
Nutritional status (weight-for-age)	
Normal	149 (57.1%)
Underweight	49 (18.8%)
Severely underweight	63 (24.1%)
Nutritional status (height-for-age)	
Normal	115 (44%)
Stunted	56 (21.5%)
Severely stunted	90 (34.5%)

STH = Soil-transmitted Helminths

Table 2 Factors Associated with STH Infections in Children Aged 24–59 Months

Risk factors	Total Examined n= 261		Bivariate analysis		
	STH (n= 30)	No-STH (n=231)	OR**	95% CI	p-value*
Age (years)					
2	11 (36.7%)	110 (47.6%)			
3	10 (33.3%)	52 (22.5%)	1.923	0.77-4.81	0.369
4	9 (30%)	69 (29.9%)	1.304	0.51-3.31	
Sex					
Male	17 (56.7%)	133 (57.6%)	1.038	0.48-2.24	1.000
Female	13 (43.3%)	98 (42.4%)			
Nutritional status (weight-for-age)					
Normal	14 (46.7%)	135 (58.4%)			
Underweight	9 (30%)	40 (17.3%)	2.170	0.87-5.38	0.231
Severely underweight	7 (23.3%)	56 (24.3%)	1.205	0.46-3.15	
Nutritional status (height-for-age)					
Normal	9 (30%)	106 (48.9%)			
Stunted	11 (36.7%)	45 (19.5%)	2.879	1.12-7.43	0.075
Severely stunted	10 (33.3%)	80 (34.6%)	1.472	0.57-3.79	
Exclusive breastfeeding practices					
<6 months	8 (26.7%)	50 (21.6%)			
6 months	19 (63.3%)	145 (62.8%)	0.819	0.34-1.99	0.651
>6 months	3 (10%)	36 (15.6%)	0.521	0.13-2.10	
Monthly income (IDR)					
500.000–2 million	19 (63.3%)	100 (43.3%)			
2–3.5 million	10 (33.3%)	107 (46.3%)	0.492	0.22-1.11	0.162
3.5–5 million	1 (3.3%)	11 (4.8%)	0.478	0.58-3.93	
>5 million	0 (0.00%)	13 (5.6%)	0.000		
Father's education					
Primary	14 (46.7%)	36 (15.6%)			
Secondary	6 (20%)	97 (42%)	0.159	0.06-0.45	0.0003
High school	8 (26.7%)	91 (39.4%)	0.226	0.87-0.59	
College	2 (6.6%)	7 (3%)	0.735	0.14-3.98	
Mother's education					
Primary	10 (33.3%)	44 (19%)			
Secondary	12 (40%)	102 (44.2%)	0.518	0.21-1.29	0.319
High school	7 (23.3%)	76 (32.9%)	0.405	0.14-1.14	
College	1 (3.3%)	9 (3.9%)	0.489	0.55-4.31	
Participation in BPJS-Healthcare program					
Yes	3 (10%)	78 (33.8%)	0.218	0.06-0.74	0.015
No	27 (90%)	153 (66.2%)			

Table 2 Continued

Risk factors	Total Examined n= 261		Bivariate analysis		
	STH (n= 30)	No-STH (n=231)	OR**	95% CI	p-value*
Water source					
Well	5 (16.7%)	43 (18.6%)			
Pump	6 (20%)	98 (42.4%)	0.527	0.15-1.82	0.015
Electric pump	19 (63.3%)	80 (34.6%)	2.043	0.71-5.85	
Spring water	0 (0%)	10 (4.4%)	0.000		
Distance between the water source and the pit latrine					
<10 m	22 (73.3%)	98 (42.4%)	0.268	0.11-0.63	0.003
>10 m	8 (26.7%)	133 (57.6%)			
Availability of household sanitation facilities					
Yes	29 (96.7%)	215 (93%)	2.158	0.28-16.89	0.721
No	1 (3.3%)	16 (7%)			
Latrine usage					
Yes	24 (80%)	191 (82.7%)	0.838	0.32-2.18	0.914
No	6 (20%)	40 (17.3%)			
Availability of septic tank					
Yes	19 (63.3%)	154 (66.7%)	0.864	0.39-1.91	0.874
No	11 (36.7%)	77 (33.3%)			
Handwashing habit					
Yes	17 (56.7%)	119 (51.5%)	1.231	0.57-2.65	0.736
No	13 (43.3%)	112 (48.5%)			
Wearing shoes/slippers in outdoors					
Yes	26 (86.7%)	203 (87.9%)	0.897	0.29-2.76	1.000
No	4 (13.3%)	28 (12.1%)			
Nail hygiene					
Clean	21 (70%)	203 (87.9%)	0.322	0.13-077	0.018
Dirty	9 (30%)	28 (12.1%)			

STH: Soil-Transmitted Helminths; OR: Odds Ratio; CI = Confidence Interval; BPJS: *Badan Penyelenggara Jaminan Sosial*; *Cochran's and Mantel Haenszel; **Chi-square analysis

stepwise procedure utilized the forward Wald method, a variable selection technique in which predictors are entered into the model based on the significance of the score statistic and removed based on the probability associated with the Wald statistic. According to the results of the multiple logistic regression test, the distance between the water source and the pit latrine OR= 0.27; 95% CI: 0.11–0.63; p=0.036), nail hygiene (OR=0.32; 95% CI: 0.13–0.76; p=0.010), and participation in BPJS-Healthcare program (OR=0.36; 95% CI: 0.15–0.91; p=0.031) were the significant factors influencing the incidence of STH infections among children aged 24 to 59 months in Bandung Regency.

Discussion

This study identified *Ascaris lumbricoides* as the most prevalent species of STH, followed by *Trichuris trichiura* and hookworms. These findings are consistent with those reported by Wang et al. in China, where the prevalence of ascariasis among preschool-aged children ranged from 4.7% to 29.5%,[11] but contrast with those of Alelign et al. in Northwestern Ethiopia,[12] where

Table 3 Multivariate Logistic Regression for STH Infections

Risk Factors	Coefficient	OR*	95% CI	p-value**
Step 1				
Distance between water source and pit latrine	-1.317	0.268	0.11-0.63	0.002
Step II				
Distance between water source and pit latrine	-1.282	0.277	0.12-0.66	0.003
Nail hygiene	-1.071	0.343	0.14-0.84	0.020
Step III				
Distance between water source and pit latrine	-1.329	0.265	0.76-0.92	0.036
Nail hygiene	-1.145	0.318	0.13-0.76	0.010
Participation in BPJS-Healthcare program	-1.010	0.364	0.15-0.91	0.031

OR: Odds Ratio; CI = Confidence Interval; *Forward-Wald; **Logistic regression analysis

hookworms were the predominant STH species. *Trichuris trichiura* had also been reported as the dominant species in other regions of Ethiopia and Côte d'Ivoire.[13,14] In Southeast Asia, *Ascaris lumbricoides* contributes the highest burden among soil-transmitted helminth infections in children, with prevalence rates ranging from 21.2% to 50% of cases.[15]

The association between potential risk factors and the incidence of STH infections was analyzed in this study. These results showed that the highest percentage of children with STH infections occurred among those whose fathers had the lowest education level, namely primary school. Meanwhile, the highest percentage of children with STH infections occurred among those whose mothers had a secondary school education. Father's education level was significantly associated with the incidence of STH infections in the bivariate analysis. Compared to fathers with only primary education, those with secondary, high school, and college education had lower odds of having children with STH infections, with odds ratios (OR) of 0.159, 0.226, and 0.735, respectively. These findings align with those reported by Pasaribu et al. in North Sumatera, where secondary and high school education levels among fathers were associated with reduced odds of STH infection, with ORs of 0.58 and 0.45, respectively.[16] On the other hand, mother's education was statistically not significant. A study conducted by Ramadhanti et al. on fathers and early childhood children in Sukamukti Village, Bandung Regency, showed that 67% of children were in the secure attachment category, 29% in the ambivalent attachment category, and 4% in the avoidant attachment category. In this pattern, fathers served as responsive, sensitive, and nurturing figures, playing a crucial role in the lives of their children.[17] Higher parental education, particularly in health-related fields, equipped children with the knowledge to promote healthy living and provide nutritious diets for their families.[18]

In addition to the role of parental education, access to healthcare services also plays a critical part in reducing the burden of infectious diseases, participation in the BPJS-Healthcare program as a form of universal health coverage for Indonesian citizens was also important to provide access to adequate healthcare and quality services, which could assist in disease prevention and management.[19] The results showed that the percentage of children infected with STH who had participated in the BPJS-Healthcare program was lower compared to children without STH infections who participated in the BPJS-Healthcare program. Participation in this program was significantly associated with the incidence of STH infections in bivariate and multivariate analyses. In addition, it was associated with an OR of 0.364 for the incidence of STH infections compared to non-participants of the BPJS-Healthcare program. This association underscores the broader importance of healthcare access in facilitating timely disease prevention and management.

However, access to healthcare alone is not sufficient to eliminate the risk of STH infections. This limitation highlights the need for a more comprehensive, integrated approach centered on water, sanitation, and hygiene (WASH). WASH strategies aimed to improve water access (quality, quantity, and distance), sanitation facilities (such as latrines and waste management), and

hygiene practices (like handwashing and safe water storage). These strategies significantly reduced the odds of STH infections rates.[20] From observations and interviews, it was found that the majority of the community had water sources. In this study, water sources typically were from dug wells or artesian water used for daily purposes. The data showed that the majority of children with STH infections use water electric pumps as their clean water source, followed by water pumps and water wells. Meanwhile, the majority of children without STH infections use water pumps as their clean water source, followed by water electric pumps, water wells, and spring water. The water source significantly influenced the incidence of STH infections in the bivariate analysis. Water sources such as water pumps, electric pumps, and spring water had OR of 0.527, 2.043, and 0.000, respectively, compared to water wells for the incidence of STH infections, which was not influenced solely by the water source. Another contributing factor was the distance between the water source and the pit latrine. The requirements for proper waste disposal included having a waste drainage system with a distance of at least 10 meters between the water source and the drainage channel.[21] Based on observations and interviews with the community, it was found that in some households, the placement of the water sources and the pit latrines was inappropriate. The percentage of children with STH infections who had a distance between the water source and the pit latrine of less than 10 m was lower than the percentage of children without STH infections who had a distance between the water source and the pit latrine of more than 10 m. Distance between the water source and the pit latrine of more than 10 m was statistically significant and associated with an OR of 0.265 for the incidence of STH infections compared to those with the distance between the water source and the pit latrine of less than 10 m. These findings are consistent with those reported by Sinaga et al. in Bandar Lampung City, which demonstrated a significant association between STH infection and both the water source and the proximity between the water source and the pit latrine. The study found that the risk of STH infection was approximately 3 times higher when the water source was contaminated and located less than 10 meters from the latrine. This could occur because the flow of water from the water source was contaminated by latrine waste. When residents use this contaminated water for washing and cooking, it could introduce microbes, including STH nematodes, into food.[22]

In addition to waterborne transmission, children are also at high risk through direct contact with contaminated environments. Children's activities often involved direct contact with dirty objects and soil contaminated with worm eggs, making it highly likely for the eggs to adhere to their hands and feet, particularly becoming lodged in the gaps between their nails. In addition, children commonly engage in hand-to-mouth behaviors, which increases their risk of ingesting contaminated soil or materials adhered to their fingers and nails, further elevating their vulnerability to STH infections.[23] This study also examined the association between nail hygiene and the incidence of STH infections. Nails were considered clean when the 5 fingers were not blackened due to dirt. Based on the observations, it was found that the number of children with STH infections who had clean fingernails and toenails was fewer compared to those without STH infections. Good nail hygiene was statistically significant and associated with an OR of 0.318 for the incidence of STH infections compared to those with bad nail hygiene. This finding is consistent with the study by Komalasari et al. in Palembang City, which reported that children with poor nail hygiene had a 3.3 times higher risk of parasitic worm infection, further highlighting nail hygiene as a critical factor in the incidence of STH infections.[24]

Other potential determinants, such as nutritional status, showed variable infection rates across different weight and height categories, but these differences were not statistically significant, contradicting the findings of some regional studies that suggested a stronger association.[1,8,9] Similarly, economic factors, breastfeeding practices, and household sanitation measures, such as latrine usage and septic tank ownership, also showed no significant statistical correlation with the incidence of STH, challenging the findings from previous studies.[12,25]

In conclusion, the father's education, BPJS-Healthcare program, access to clean water sources, distance between water sources and pit latrine, and nail hygiene were statistically significant factors influencing the incidence of STH infections among children aged 24 to 59 months. The findings highlight the importance of addressing both environmental and behavioral determinants in reducing the burden of STH infections. Specifically, promoting good nail hygiene and improving access to clean water, alongside behavioral interventions targeting hand-to-mouth activities in children, are crucial

for effective prevention. Public health strategies should prioritize educational campaigns, particularly targeting fathers, to foster better hygiene practices and improve sanitation conditions.

This study has several limitations. The use of self-reported data through questionnaires may have introduced recall bias, as participants could have provided socially desirable responses. In addition, the sample size, while adequate for the study's scope, may limit the statistical power and generalizability of the results, particularly given the large target population. Future studies with larger samples and longitudinal approaches are recommended to validate these findings and explore causal pathways more thoroughly.

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Correlation Between ERCP Implementation Time and Outcomes of Patients with Acute Cholangitis Due to Choledocholithiasis

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Abstract

Acute cholangitis is a serious condition, and timely ERCP is essential for effective management. This prospective cohort study was conducted at Dr. Hasan Sadikin Hospital in Bandung, Indonesia, from June 2023 to April 2024, to evaluate the feasibility of performing ERCP beyond 48 hours in the presence of various limitations. The study included patients with acute cholangitis caused by common bile duct stones. The ERCP timing was categorized into three groups: less than 48 hours, 48-72 hours, and more than 72 hours. Outcomes measured were hospital stay duration, ICU admission, and 30-day mortality. Of these patients, 52.8% underwent ERCP at 48-72 hours, 27.8% after 72 hours, and 19.4% before 48 hours. The median hospital stay was 7.5 days (IQR 3-15). ICU admissions occurred only in patients receiving ERCP after 72 hours (30.0%), a significantly higher rate compared to the other groups (p=0.014). A strong correlation was found between delayed ERCP and longer hospital stays (r=0.711, p<0.01), as well as ICU admission (r=0.405, p=0.014), though no significant correlation with mortality was observed (r=-0.021, p=0.905).

Keywords: Acute cholangitis, biliary drainage, choledocholithiasis, duration of surgery, endoscopic retrograde cholangiopancreatography

Introduction

Acute cholangitis, also known as ascending cholangitis, is a serious illness that affects the entire body. It presents with a set of symptoms including fever, jaundice, and abdominal discomfort (known as Charcot's triad). This condition can be life-threatening, with a historically documented fatality rate of over 50%.[1] Acute cholangitis, also known as angiocholitis, is a highly infectious condition that affects the bile and bile ducts. The discovery of this ailment can be attributed to Jean Martin Charcot (1825–1893) in 1877.[2] Two choledocholithiasis is believed to be the cause in 28-70% of cases of acute cholangitis. The symptoms of this condition include abdominal pain, jaundice, fever, and hepatomegaly.[3]

Studies indicate that the occurrence of acute cholangitis varies between 0.3% and 1.6%, with approximately 12.3% of cases being classified as severe cholangitis.[4] Four Research conducted in the United States revealed that choledocholithiasis is present in approximately 10% to 15% of the Caucasian population in the country. Among patients with choledocholithiasis who present to the emergency room, around 6% to 9% receive a diagnosis of acute cholangitis. The study reported that there were no notable gender disparities, and the majority of participants were between the ages of 50 and 60. Acute cholangitis affects around 200,000 individuals annually in the United States.[5] There is a lack of available data on the occurrence of acute cholangitis in Indonesia or specifically in the region of West Java.

The management of acute cholangitis, as outlined in several studies and the 2018 Tokyo Guidelines, necessitates prompt intervention to address the two primary issues: infection and obstruction of the biliary path. Regarding infection, prompt treatment is administered, specifically by fluid resuscitation and antibiotics.

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Elevated intraductal pressure hinders the release of antibiotics from the biliary path, necessitating the use of biliary drainage to address this form of obstruction. The Tokyo Guidelines 2018 suggest that immediate biliary draining should be performed for cases of moderate and severe cholangitis. Biliary drainage is indicated in cases of mild acute cholangitis only when there is no response to antibiotic treatment.[6]

Endoscopic retrograde cholangiopancreatography (ERCP) is a specialized operation that uses an endoscope to diagnose and treat abnormalities of the pancreatic and biliary systems. This process was created as a diagnostic method in the late 1960s and early 1970s. The inaugural biliary sphincterotomy took place in 1974, and since then, ERCP has had swift advancement as a therapeutic intervention for the biliary tract. ERCP is a superior alternative to PTBD (percutaneous biliary drainage) due to its lower invasiveness, enhanced safety, ability to be performed at the patient's bedside, effectiveness in removing bile duct stones, independence from the need for coagulopathy correction, and the option to avoid radiation exposure if required (such as in pregnant patients). ERCP has a better success rate compared to PTBD. Percutaneous transhepatic biliary drainage (PTBD) is typically carried out in individuals who do not respond to initial endoscopic retrograde cholangiopancreatography (ERCP) or if there is aberrant anatomy caused by past surgical interventions.[7]

The ERCP surgery commences by inserting the duodenoscope through the oral cavity. The duodenoscope subsequently traverses the pylorus of the stomach and enters the duodenal bulb. The wire-guided procedure is advised to be performed under fluoroscopy, with the wire being inserted into either the bile duct or pancreatic duct prior to injecting contrast. The European Society of Gastrointestinal Endoscopy (ESGE) advises the utilization of 5-Fr stents, which are shorter in size, instead of 3-Fr stents, for individuals at high risk. Stents that are kept in place should be taken out within a maximum of 10 days after they are inserted.[8]

According to the 2018 Tokyo guidelines, a quick implementation of ERCP is associated with improved patient outcomes. The optimal timeframe for implementing ERCP in individuals with acute cholangitis remains a topic of active debate. Nevertheless, the consensus among the majority of experts is that biliary decompression should be carried out within a 48-hour timeframe. Retrospective studies examining the most favorable timing for ERCP and early drainage (within 24-48 hours of cholangitis onset) have shown a decrease in the occurrence of organ failure, shorter hospital stays, and lower mortality rates compared to delayed drainage. The majority of patients experienced benefits from receiving immediate drainage within 24 hours.[6] A meta-analysis study yielded consistent results when ERCP was conducted within a 48-hour timeframe.[9] A study conducted by Khamaysi and Taha in Turkey demonstrated that doing ERCP within 12 hours can significantly decrease the death rate at day 30.[10] The current European recommendations also advise performing ERCP within 12 hours for patients experiencing septic shock.[11] Hou et al's findings diverged from previous studies, as they concluded that the timing of ERCP implementation had no impact on adverse events or the success of ERCP procedures.[12]

At Dr. Hasan Sadikin General Hospital (RSHS), ERCP has become a routine procedure for managing cholangitis, guided by TG18 recommendations. However, challenges remain, including limited ERCP-trained staff and equipment availability, making procedures within 24 hours unfeasible. In this context, there is a lack of local data assessing the impact of ERCP timing on patient outcomes in Indonesia. Costs related to the ERCP procedure might be covered by private or governmental insurance, such as National Health Insurance (BPJS), which aids in the treatment process. The implementation of the ERCP procedure at RSHS faces the challenge of a limited number of nurses having the expertise and skills required to assist during the surgery.. Therefore, this study aims to evaluate the relationship between ERCP timing—specifically procedures performed within 48 to 72 hours—and clinical outcomes in patients with acute cholangitis at Dr. Hasan Sadikin General Hospital, Bandung.

Methods

This prospective cohort study was conducted from June 2023 to April 2024 at Dr. Hasan Sadikin General Hospital, Bandung. The study population consisted of patients diagnosed with acute cholangitis at RSUP Dr. Hasan Sadikin between June 2023 and April 2024. The study included patients who met the following criteria: (1) age >18 years, (2) diagnosis of Choledocholithiasis with Acute Cholangitis, and (3) underwent

ERCP. The exclusion criteria for this study are as follows: (1) Patient refusal to provide informed consent to participate as a research subject, and (2) Patient history of malignancy. The exclusion criteria for this trial were: (1) The patient was compelled to leave the study or declined to have ERCP during the designated period. Sampling was performed using a total sampling method. The samples utilized by researchers included only of cases of Choledocholithiasis complicated by acute cholangitis at Dr. Hasan Sadikin Bandung. Data were analyzed using Microsoft Excel 2027 and SPSS version 25.0. Descriptive analysis will provide a detailed account of the specific attributes of every patient. The data will be displayed as frequency (n) and percentage (%) for categorical variables, and as average and standard deviation for continuous variables. Patients will be categorized based on the timing of ERCP implementation into three groups: those who receive ERCP within 48 hours, those who receive it between 48 and 72 hours, and those who receive it beyond 72 hours. Subsequently, a bivariate analysis was conducted. Bivariate analysis is a statistical analysis conducted to examine the correlation between two variables. This study employed bivariate analysis using the chi-square test to examine the link between the timing of ERCP implementation and the outcome of patients with acute cholangitis. Ethical approval was obtained from the Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital (No. 1B.02.01/X.6.5/152/2023).

Results

This prospective cohort study was conducted at Dr. Hasan Sadikin General Hospital, Bandung, from June 2023 to April 2024, involving patients with choledocholithiasis and acute cholangitis. A total of 36 patients met the inclusion criteria and were included in the analysis. Data collected included demographic characteristics, history of cholangitis, severity of cholangitis (Tokyo Guidelines), ERCP timing, and laboratory findings. Patient characteristics are shown in Table 1.

The mean age of participants was 45.36±15.02 years. Most were female (58.3%). All patients had a history of cholangitis. Based on Tokyo Guidelines 2018,55.6% had Grade I cholangitis, 36.1% Grade II, and 8.3% Grade III When considering the timing of ERCP, it is observed that the majority of patients, specifically 52.8%, underwent the surgery between 48–72 hours of diagnosis. This was followed by 27.8% of patients who had the treatment more than 72 hours after diagnosis, and 19.4% of patients who had it within less than 48 hours.

The clinical characteristics of the research subjects were assessed using the timing of the ERCP operation, specifically the implementation method within several time frames: less than 48 hours, 48-72 hours, and more than 72 hours after the diagnosis was made. This information is presented in Table 2. Based on this investigation, numerous inferences can be inferred. The research revealed that there was no statistically significant variation in the average age among the various ERCP timing groups. This indicates that age does not play a decisive role in the selection of ERCP timing. Regarding gender, there was no notable disparity in the distribution of male or female patients across the three groups. This demonstrates that the timing of ERCP does not have an impact on the gender distribution of individuals who undergo the surgery. Based on the results obtained from 3 patients diagnosed with grade 3 cholangitis, it was found that 2 patients (10.5%) underwent ERCP within 48-72 hours, 1 patient (10.0%) underwent ERCP after 72 hours, and no patient underwent ERCP within 48 hours. According to the 2018 Tokyo

Table 1 Subject Characteristics

Variable	Total (n=36)
Age (year)	45.36±15.02
Sex (%)	
Male	15 (41.7%)
Female	21 (58.3%)
Grade of Cholangitis (%)	
Grade 1	20 (55.6%)
Grade 2	13 (36.1%)
Grade 3	3 (8.3%)
ERCP Implementation Time (%)	
<48 hours	7 (19.4%)
48–72 hours	19 (52.8%)
>72 hours	10 (27.8%)

Note: Categorical data is typically provided in terms of numbers or frequencies, as well as percentages. On the other hand, numerical data is usually presented using measures such as mean, median, standard deviation, and range. ERCP=Endoscopic Retrograde Cholangiopancreatography.; SGOT=serum glutamic oxaloacetic transaminase;

Table 2 Comparison of Characteristics Across ERCP Timing Groups

Variable	<48 hours (n=7)	48-72 hours (n=19)	>72 hours (n=10)	p-value
Age (year)	48.43±14.60	44.95±16.03	44,00±14.54	0.832
Sex (%)				0.992
Male	3 (42.9%)	8 (42.1%)	4 (40.0%)	
Female	4 (57.1%)	11 (57.9%)	6 (60.0%)	
History of cholangitis (%)				1.000
Yes	7 (100%)	19 (100%)	10 (100%)	
No	-	-	-	
Grade of cholangitis (%)				0.329
Grade 1	5 (71.4%)	12 (63.2%)	3 (30.0%)	
Grade 2	2 (28.6%)	5 (26.3%)	6 (60.0%)	
Grade 3	-	2 (10.5%)	1 (10.0%)	

Guideline, the recommended treatment for grade 3 cholangitis is urgent biliary drainage, which necessitates ERCP to be performed within 24 hours. However, at RSUP Dr. Hasan Sadikin, the timing of ERCP differs from the guideline. Due to limited human resources, it is now not possible to do ERCP within 24 hours. Additionally, the prompt execution of ERCP at grade 3 is hindered by the Anesthesia department's evaluation of anesthesia risks, since the patient's condition is more unstable at this stage. In general, there were no notable variations in clinical characteristics between the three groups categorized by the date of ERCP. These findings indicate that the three groups have similar patient features, allowing for the analysis to progress to the next stage.

Table 3 provides a description of the results obtained from the research participants, categorized according to the timing of the ERCP surgery. Various inferences can be derived from this table. In this study, the patients had a median length of stay of 7.5 days, with an interquartile range of 3-15 days. The length of stay varied significantly between the groups of patients who had ERCP operations more than 72 hours and less than 48 hours after diagnosis (15.5 [IQR 14–25.5] vs. 3 [IQR 2–3] days; p<0.01) (Figure

Table 3 Outcomes Based on ERCP Timing Group

Variable	Total (n=36)	<48 hours (n=7)	48-72 hours (n=19)	>72 hours (n=10)	p-value
Length of stay (days)	7.5 (3–15)	3 (2–3)	7 (3–11)	15.5 (14–25.5)	<0.01*
Grade I	4 (3–9.75)	3 (3–3)	4 (2.75–7.25)	15 (15–21)	0.453
Grade II	11 (9–16)	3 (2.25–2.75)	9 (9–11)	16 (15.25–22.75)	0.018*
Grade III	10 (8.5–25)	–	24 (7–40)	10 (N/A)	1.00
ICU admission (%)	3 (8.3%)	–	–	3 (30.0%)	0.014*
Grade I	1 (5.0%)	–	–	1 (33.3%)	0.051
Grade II	2 (15.4%)	–	–	2 (33.3%)	0.252
Grade III	–	–	–	–	
Mortality (%)	1 (2.8%)	–	1 (5.3%)	–	0.631
Grade I	–	–	–	–	
Grade II	–	–	–	–	
Grade III	1 (33.3%)	–	1 (50.0%)	–	0.386

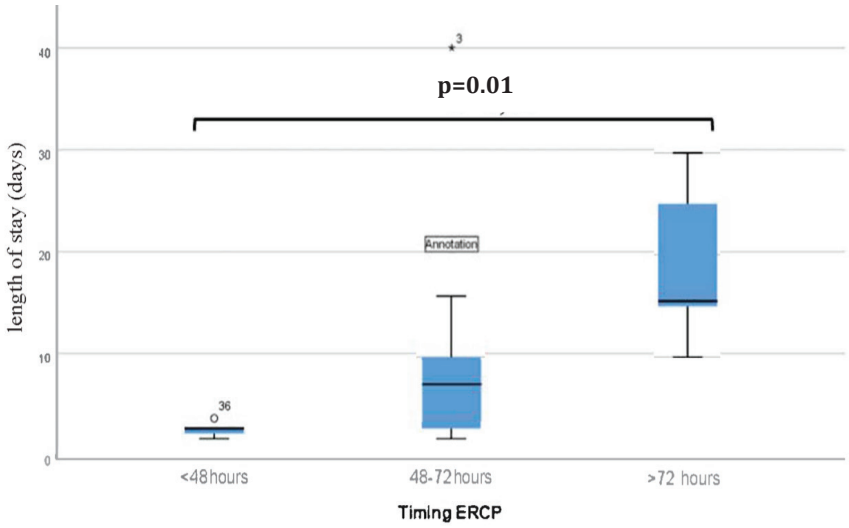

Figure 1 Comparison of Length of Stay by ERCP Timing Group

1). Out of the patients who underwent the ERCP surgery, 3 individuals (8.3% of the total) were hospitalized to the Intensive Care Unit (ICU). 30.0% of these patients were part of the ERCP timing group that exceeded 72 hours, which was substantially more than the ERCP timing groups that were less than 48 hours and between 48 and 72 hours (P=0.014). One patient, accounting for 2.8% of the total, died following the ERCP operation in this study. The patients included in the ERCP timing group of 48-72 hours accounted for 5.3% of the total. Nevertheless, the analytical findings indicated that there were no substantial disparities among the three groups (p=0.631).

This study further categorized each outcome based on the Grade of cholangitis. Patients with cholangitis Grade I had a median stay of 4 days with interquartile range of 3-9.75 days. Patients with cholangitis grade II had a median stay of 11 days (IQR: 9-16) and patients with cholangitis Grade III had a median stay of 10 days (IQR: 8.5-25). Interestingly, in patients with grade II cholangitis, the timing of ERCP was significantly associated with the duration of hospital stay (p=0.018). Patients with grade I cholangitis had a 5% rate of ICU admission, which was one patient admitted after ERCP procedure within >72 hours. While patients with grade II cholangitis had a 15.4% rate of ICU admission in which two of them are admitted into the ICU after ERCP procedure within >72 hours. However, both of them are not statistically significant (p=0.051 and p=0.252, respectively). Ultimately, performing ERCP more than 72 hours after diagnosis is associated with a longer hospital stay and a higher rate of intensive care unit (ICU) admission compared to procedures performed within 72 hours.

The analysis aims to establish the correlation between an independent variable, specifically the timing of ERCP, and a dependent variable, which encompasses the length of stay, ICU admission, and mortality. Based on the statistical analysis using the Spearman correlation test, numerous conclusions can be derived in accordance with Table 4. The link between the ERCP timing variable and length of stay was shown to be statistically significant, with a P value of <0.01. The correlation coefficient (r) is

Table 4 Correlation between ERCP Timing and Outcomes

Variable	Correlation Coefficient (r)	p-value
Length of Stay (days)	0.711	<0.01*
ICU Admission	0.405	0.014*
Mortality	-0.021	0.905

Note: Spearman's correlation test. *p<0.05 indicates statistical significance

0.711, indicating a strong positive association. Between the temporal variable of ERCP and ICU admission, the correlation had a p-value of 0.014, indicating a statistically significant link. The correlation coefficient (r) of 0.405 suggests a positive association with moderate strength. The association between the temporal variable of ERCP and death was assessed, yielding a P value of 0.905. This result indicates that there is no significant or statistically meaningful correlation between the two variables. The correlation coefficient (r) obtained is -0.021, indicating a weak negative connection that can be disregarded due to its minor strength.

In summary, delayed ERCP implementation beyond 72 hours was significantly associated with prolonged hospitalization and higher ICU admission rates. However, no significant association was observed between ERCP timing and mortality, likely due to the low number of events. These findings suggest timely ERCP may improve short-term outcomes in acute cholangitis patients.

Discussion

Acute cholangitis is a severe infection of the bile and bile ducts that is life-threatening. It is characterized by the presence of fever, jaundice, and stomach discomfort, which together are known as Charcot's triad. The death rate for this condition can exceed 50%.[2] Choledocholithiasis has been found to be present in 28-70% of cases.[3] Acute cholangitis is a condition characterized by inflammation of the bile ducts.

According to the Tokyo Guidelines 2018 early biliary drainage through ERCP is a recommended approach to manage the infection and relieve obstruction. However, there remains debate regarding the optimal timing of ERCP. The recommendations also advise that performing ERCP at an earlier stage will result in a more favorable outcome for the patient.[13,14] However, there is still ongoing disagreement regarding the optimal timing for performing ERCP in individuals with acute cholangitis.

This research is the first in Indonesia to evaluate the relationship between ERCP timing and clinical outcomes in patients with acute cholangitis secondary to choledocholithiasis. Findings indicate that delayed ERCP (>72 hours) is significantly associated with prolonged hospital stay and increased ICU admission. The study cohort predominantly consisted of females (58.3%), with a mean age of 45.36 ± 15.02 years.

Similarly, Similarly, Lee et al. reported 55% female patients with a mean age of 59 ± 19 years, with no significant differences between early and delayed ERCP groups.[15] In line with previous studies conducted by Parikh et al., Patel et al., and Mulki et al., who also reported that females constituted more than 50% of patients across all ERCP timing groups.[16-18] On et al. also observed a higher proportion of females (55.8%) in their study.[19] When assessing the severity of cholangitis based on the Tokyo Guideline classification, grade 1 was the most common grade in our study cohort, accounting for 5.6% of patients. There was no significant difference observed between the ERCP timing groups in terms of the distribution of cholangitis grades. Similarly, On et al. reported that 51.3% of their patients were categorized as having mild acute cholangitis, while 32.6% had moderate grade and 16.1% had severe grade. Furthermore, there was no discernible variation in the duration of ERCP (endoscopic retrograde cholangiopancreatography) among patients with acute cholangitis caused by choledocholithiasis, regardless of the severity classification.[19]

Grade 1 cholangitis is defined by the lack of distinct features seen in grades 2 and 3 cholangitis. Grade 2 cholangitis can be diagnosed based on the presence of fever, a white blood cell counts higher than 12,000 cells/mm^3 or lower than 4000 cells/mm3, bilirubin levels over 5 mg/dL, low albumin levels, or advanced age. Within this study sample, 13 patients (36.1%) were categorized as grade 2. Three of our patients (8.3%) were diagnosed with grade 3 cholangitis, characterized by the presence of end-organ destruction. Assessing the gravity of cholangitis is crucial as it will aid in determining the subsequent therapeutic approach. For moderate and severe cases of illness, it is imperative to promptly perform biliary drainage. Meanwhile, patients with moderate acute cholangitis typically only require antibiotic therapy. Biliary drainage will be explored if the patient does not show improvement with antibiotic therapy. Most patients with acute cholangitis caused by choledocholithiasis will often respond to initial conservative therapy, especially those with mild instances. Nevertheless, approximately 15% of these patients may potentially undergo deterioration to the extent of sepsis, a condition linked to significant morbidity and mortality. Thus, it is crucial for patients to undergo regular reassessment using the most up-to-date Tokyo Guideline criteria. This assessment should be conducted immediately after the diagnosis, within 24 hours of the diagnosis, and within the

24-to-48-hour timeframe. This will allow for timely identification of patients who were initially diagnosed with mild cholangitis but later meet the criteria for moderate or severe cholangitis, thus requiring treatment. Timely biliary decompression and avoiding unnecessary delays in doing ERCP can help prevent complications.[2]

Patients who received the ERCP surgery more than 72 hours after diagnosis had a substantially longer hospital stay compared to those who received the treatment within 48 hours (15.5 [IQR 14–25.5] vs. 3 [IQR 2–3] days; p<0.01). The correlation analysis revealed a statistically significant and high positive connection (r=0.711; P<0.01) between the timing of ERCP and the length of stay. Furthermore, we measured a subgroup analysis based on cholangitis severity. It was found that in patients with grade II cholangitis, the timing of ERCP was significantly correlated with the duration of hospital stay (p = 0.018). This finding indicated that prompt intervention may be particularly effective in this population. This trend was not evident in grade I or III, likely due to variations in baseline severity or a small sample size. These results are consistent with multiple findings from prior research. In a retrospective study, Khashab et al. found that when ERCP procedures were delayed by more than 72 hours after admission, it led to longer hospital stays (odds ratio [OR] = 19.8; P = 0.008) and higher inpatient costs (OR=11.3; P = 0.03).[20] Another study by Navaneethan et al. also showed that when the time from arrival to ERCP exceeded 72 hours, it resulted in a 70% increase in the average length of hospital stay (P < 0.01).[21] In a retrospective assessment conducted by Chak et al, individuals with cholangitis who received Early ERCP (within 24 hours of hospital admission) had a considerably shorter hospital stay compared to those who underwent delayed ERCP (median 4 vs 7 days; P<0.005).[22] In Zhu et al.'s report, it was found that a delay of 1 day in the Biliary draining treatment results in an increase of 1.49 days in the length of hospital stay (p<0.0001). There was a significant correlation between delayed biliary drainage (>48 hours) and a longer stay in the intensive care unit (ICU) (p=0.0096).[23]

In the study conducted by Patel et al., a variation in the duration of hospitalization was observed. Patients who underwent ERCP within 24 hours had a hospital stay of 7 days, while those who had the procedure between 24-48 hours stayed for 6 days, and those who had it after 48 hours stayed for 14 days.[17] A similar trend was observed in another study conducted in Thailand, where ERCP performed within 48 hours was associated with a higher median length of hospital stay. The duration of hospitalization was significantly shorter in the group with a 6-day average compared to the group with an 11-day average (p < 0.01).[24] In a study conducted by Parikh et al. comprising 107,253 patients, the group that received ERCP after 48 hours had the longest hospital stay and incurred the highest costs compared to other groups (p<0.01).[16] A study conducted by Aboelsoud et al. found that patients who underwent ERCP within 24 hours had shorter hospital stays (7.71 vs. 13.57 days, p=0.01) and ICU stays (3.25 vs. 4.95 days, p=0.040).[6] Another study by Mulki et al. showed that the mean length of stay was longer in the group that underwent ERCP after 48 hours compared to the group that underwent early ERCP (6.9 days vs. 4.5 days, p<0.01).[18]

Lyu et al. conducted a systematic review and meta-analysis involving 7 observational studies with a total of 88,562 patients with acute cholangitis. The study found that performing ERCP within 24 or 48 hours after admission was linked to a shorter hospital stay (p<0.01).[13] Another meta-analysis by Iqbal et al. involving 9 observational studies and a total of 7,534 patients also showed that patients who underwent ERCP within 48 hours had a significantly lower length of stay, with a mean difference of 5.56 days (95% CI: 1.59–9.53).[9] The longer hospital stay in patients with delayed ERCP can be attributed to the time spent managing the underlying decompensated condition.[17] These findings emphasize that decisive treatment not only improves patient outcomes, but also reduces the expenditures associated with hospitalization for cholangitis.[18]

In this study, the group of patients who underwent ERCP after 72 hours had the greatest rate of admission to the intensive care unit compared to the other groups (30% vs. 0% vs. 0%; p=0.014). The correlation analysis revealed a statistically significant moderate positive association (r=0.405, p=0.014) between the date of ERCP and the likelihood of being admitted to the ICU. Khashab et al. discovered in their prior study that delays of 72 hours in ERCP were linked to worse clinical outcomes, including the need for ICU admission.[20] The prolongation of hospitalization and transfer to the intensive care unit (ICU) may also be linked to a heightened likelihood of enduring organ dysfunction in patients. who had a delayed endoscopic retrograde cholangiopancreatography (ERCP) procedure. In retrospective research conducted

by Lee et al., delayed endoscopic retrograde cholangiopancreatography (ERCP) was defined as ERCP performed at least 48 hours after hospitalization. The researchers discovered that the delay in ERCP was linked to a higher occurrence of persistent organ failure more than 48 hours after admission. Persistent organ failure was defined as a ≥1.5-fold increase in creatinine levels from baseline to ≥1.5 mg/dL, or the need for dialysis, mechanical ventilation, and/or vasopressors to treat hypotension. The odds ratio for this association was 3.1, with a 95% confidence interval of 1.4–7.0. Furthermore, the study revealed that for every 1-day delay in ERCP, there was a 17% higher risk of persistent organ failure (95% CI: 5-29%). Another study conducted by Boender et al., which included 95 patients with acute cholangitis caused by choledocholithiasis, also demonstrated that a delay in ERCP (>3 days) was linked to a greater likelihood of complications and morbidity.[38]

Cholangitis, being a systemic condition, often leads to the occurrence of persistent organ failure and multiorgan failure.[15] Increased pressure in the bile ducts can lead to the breakdown of tight connections between liver cells, allowing germs to enter the bloodstream and cause sepsis.[20] Research has demonstrated that performing biliary decompression can effectively decrease the occurrence of cholangiovenous reflux, leading to a subsequent reduction in levels of bile and serum endotoxins. This process of decompression can also enhance the elimination of IgA and antibiotics via bile excretion. In theory, implementing early biliary drainage could enhance organ function and reduce hospitalization duration.(6) However, our study did not consider persistent organ failure as one of the variables examined. Therefore, we cannot definitively determine its possible impact as a confounding factor in the study's conclusions.

The study found that the in-hospital death rate was 2.8% among patients who underwent ERCP between 48 and 72 hours. The analytical results indicated that there were no statistically significant differences seen among the three ERCP timing groups (P=0.631). Similarly, in the examination of correlation, there was no statistically significant association seen between the two values (r=-0.021; P=0.905). Similarly, a multi-center observational research undertaken by Kiriyama et al. in Japan and Taiwan found that performing ERCP within 24 or 48 hours following hospital admission did not result in better 30-day death rates compared to performing ERCP at a later time.[22] Zhu et al. discovered that there was no notable rise in in-hospital mortality (OR=1.03; 95% CI 0.93–1.13) or 30-day mortality (OR=1.01; 95% CI 0.87–1.14) when patients with severe acute cholangitis underwent delayed biliary drainage.[23] A study conducted by Patel et al. found that the timing of ERCP did not have an impact on mortality, regardless of the severity of the cholangitis.[17] In another study by Aboelsoud et al., there was a slight decrease in in-hospital mortality in the group that underwent ERCP within 24-48 hours, but this difference was not statistically significant (OR = 0.47; 95% CI: 0.17–1.29; p = 0.146).[6] Similar results were reported by Khamaysi and Taha, with a 30-day mortality rate of 15% in the group that underwent ERCP within 12 hours and 21%.[10] Inamdar et al. found no significant difference in hospital mortality between patients treated on weekdays and those treated on weekends. However, it was observed that a higher number of patients treated on weekdays underwent ERCP within 48 hours compared to patients treated on weekends in this study. The weekend mortality rate was 70% compared to 65.4%, with a p-value of less than 0.01.[11] In another study by Athigakunagorn et al., there was no significant difference in overall mortality rate between the group who received early ERCP (<48 hours) and the group that received delayed ERCP.[24]

These data align with our own, providing support for the concept that promptly conducted biliary drainage may not be linked to reduced mortality. Indeed, it is believed that impulsive actions can heighten the risk of anesthesia and trigger temporary bacteremia, which may result in clinical deterioration.[23] In a study conducted by Huang et al., it was discovered that the group of patients who underwent ERCP within 24 hours had a notably higher rate of admissions to the intensive care unit (ICU). The difference in percentages was statistically significant, with a substantially greater rate of 11.2% compared to 4% (p=0.01).[23] Therefore, it may not be crucial for patients with severe acute cholangitis to undergo biliary decompression as early as possible. Instead, it can be safely performed after administering antibiotics, ensuring adequate resuscitation, and stabilizing organ function within 24 hours.[23] According to Jang et al., urgent ERCP (≤24 hours) can be considered for patients with grade 1 or 2 acute cholangitis as it can reduce the duration of hospitalization.[24] However, due to the limited number of patients with severe cholangitis, our study did not conduct an analysis stratified by the severity of the condition. Therefore, we cannot draw a

conclusion based on this.

Nevertheless, multiple prior research have discovered advantages in relation to mortality when ERCP implementation time is expedited. According to Navaneethan et al., they found that a door to ERCP time longer than 72 hours was independently linked with a higher 30-day mortality rate (OR=3.36; 95%CI: 1.12-10.20). Another study, which included 166 patients with Acute cholangitis, demonstrated that performing early ERCP (within 24 hours) resulted in a significant reduction in death within 30 days. The odds ratio (OR) was 0.23, with a 95% confidence interval (CI) of 0.05-0.95, and a p-value of 0.04.[25] In a comprehensive study conducted by Parikh et al., they examined a large database of patients with acute cholangitis caused by choledocholithiasis. The study found that the highest risk of in-hospital mortality was observed in patients who did not undergo ERCP. The second highest risk was seen in patients who underwent ERCP after 48 hours (P<0.01).[16] Furthermore, a comprehensive analysis conducted by Seo et al. discovered that performing ERCP more than 72 hours after admission was linked to a higher death rate (odds ratio 1.80; P<0.01). The same result was found in the study conducted by Lyu et al.[25] A meta-analysis has found that performing ERCP within 48 hours of hospital admission is associated with lower in-hospital and 30-day mortality rates (p<0.01 and p=0.03) compared to performing ERCP more than 48 hours after admission.[13] Another meta-analysis by Iqbal et al. has also shown that patients who undergo emergency ERCP within 48 hours have a reduced risk of organ failure (OR 0.69; 95% CI: 0.33–1.46) and 30-day mortality (OR 0.39; 95% CI: 0.14–1.08).[9] Therefore, the findings of this study cannot be directly concluded. In addition, due to the limited size of this study sample, particularly in the group that underwent ERCP within 48 hours, and with just 1 patient experiencing mortality as the outcome, the statistical power is diminished.

Prior research has demonstrated that timely identification of cholangitis and increased utilization of biliary drainage can effectively decrease the overall mortality rate linked to acute cholangitis.[13] Several prior guidelines have also specified the precise timing of endoscopic biliary drainage for individuals with acute cholangitis. Current guidelines provide varied recommendations on ERCP timing. The European Society of Gastrointestinal Endoscopy recommends performing ERCP within 12 to 72 hours based on severity, while the Tokyo Guidelines 2018 define urgent ERCP as within 24 hours and early ERCP as 24-48 hours post-admission.[12] In practice, early ERCP within 24 hours remains difficult to implement at Dr. Hasan Sadikin General Hospital Bandung due to staffing limitations.

This study has limitations, including its non-randomized observational design, small sample size, single-center setting, and short follow-up duration confined to in-hospital mortality. The inability to control for confounding variables further restricts generalizability. Future research should employ larger, multicenter cohorts and examine long-term outcomes of ERCP timing.

In conclusion, delayed ERCP beyond 72 hours is significantly associated with prolonged hospitalization and increased ICU admission, suggesting that earlier ERCP may yield better outcomes. No significant association was found between ERCP timing and in-hospital mortality; however, this should be noted since our study has limited sample sizes. Furthermore, multi-center studies with larger sample sizes are required to support these findings and analyze the effects of ERCP timing on various severity grades of cholangitis.

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