RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n3.3066
Majalah Kedokteran Bandung. 2023;55(3):171–175

Majalah Kedokteran Bandung (MKB)

Received: October 23, 2023
Accepted: May 2, 2023
Available online: September 30, 2023

AST to Platelet Ratio Index (APRI), Fib-4 Score, and Pregnancy Outcome of Pregnant Women with Hepatitis B

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Abstract

Hepatitis B virus infection in pregnancy has become a major concern in many developing countries. The relationship between hepatitis B virus infection and pregnancy is complex and puzzling. This study aimed to investigate the relationship between hepatitis B virus infection and pregnancy outcomes with the insights into the AST to Platelet Ratio Index (APRI) and Fib-4 score. This was a cross-sectional study on pregnant women with hepatitis B virus infections who underwent labor at dr. Zainal Abidin Hospital General Hospital, Aceh, Indonesia. Data were collected from the obstetric ward patient medical records from 2017 to 2019 and 77 pregnant women was identified to be infected with hepatitis B virus, of which 44 had complete medical record data and were included in the analysis. The median APRI in this study was 0.30 (0.1–1.2) while the median FIB-4 score was 0.74 (0.3-1.9). Delivery with live births was identified in 42 (95.5%) women. Term pregnancy and vaginal delivery were observed in 39 (88.6%) and 10 (22.7%) women, respectively, Complicated pregnancy was seen in 14 (31.8%) of pregnancies that included complications such as oligohydramnios, HELLP, severe preeclampsia, placenta previa, and premature rupture of membranes. APRI was higher in the stillbirth group (0.5 [0.2–0.8] p=0.682) and preterm birth group (0.4 [0.2–0.6], p=0.502). FIB-4 scores were higher in the stillbirth group (1.2 [0.5–1.8], p=0.517) and preterm birth group (0.9 [0.4–1.9], p=0.529). Hence, pregnancy does not always worsen liver function and is not related to the natural course of hepatitis B infection. Pregnancy with hepatitis B without fibrosis is not associated with poor pregnancy outcomes. Routine liver function examination is needed in pregnant mothers with hepatitis B virus infections.

Keywords: Fibrosis, hepatitis B, pregnancy

Introduction

Hepatitis B Virus (HBV) infection in pregnancy has become a serious issue that needs to be addressed in developing countries. HBV infection common signs include mild diarrhea, anorexia, nausea, vomiting, malaise, and abdominal pain in the gravid uterus. These signs are quite similar to pregnancy signs. Therefore, diagnosing HBV infection in pregnancy may be challenging. Women should receive hepatitis B surface antigen (HBsAg) at their first prenatal or trimester visits. Later, they should be tested again during pregnancy if necessary. Thus, for diagnosis purposes, the presence of HBsAg in the bloodstream remains the serological hallmark.[1,2]

Understanding the relationship between HBV infection and pregnancy is not easy. It is important to have a good analysis of maternal HBV infection impacts on pregnancy outcomes, particularly in the regions where the cases are prevalent. Even though most studies confirm pregnancy does not necessarily harm the function of the liver, other research suggests that it may affect the liver size and blood flow and be associated with significant gestational and fetal complications.[1,3–5]

In China, the prevalence of HBV infection among women of reproductive age may reach 2-8%, whereas it is only 0.4% in the USA. The majority of HBV-infected pregnant women are chronic carriers, as shown by a positive serum HBsAg status.[6] Prevalence of HBV infections in Indonesia was 0.2% in 2013 and 0.4% in 2018,

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while in Aceh, 0.7% in 2013 and 0.4% in 2018.[7]

While some studies suggest that maternal HBV infection, either symptomatic, asymptomatic, or cirrhosis, may be linked to unfavorable pregnancy outcomes like gestational diabetes, antepartum hemorrhage, birth abnormalities, stillbirths, and preterm delivery,[5,8,9] other studies agree that HBV infection during pregnancy is not linked to poor pregnancy outcomes similar to the general adult population.[1,10–12]

There have been noninvasive methods used to assess liver fibrosis in the past. A combination of two independent noninvasive tests, two or a blood test plus Elastography, can be employed. However, no single noninvasive test or model has been created yet to match the information gained from actual histology. An easy-to-use and accurate test for identifying severe fibrosis has been reviewed as the aspartate aminotransferase (AST) to platelet ratio index (APRI).[13] In individuals with chronic liver disease, an APRI score was first described in 2003. It was found to be positively connected with fibrosis..[9,13] APRI and fibrosis -4 (FIB-4) were positively correlated with the fibrosis score.[14]

Methods

This observational analytic study took place at Dr. Zainoel Abidin teaching hospital Banda Aceh, the major tertiary health institution in Aceh, within three years with a cross-sectional design. Data was taken from obstetric ward patients' medical records from 2017 to 2019. This study used a total sampling of all HbsAg-positive pregnant women who underwent delivery at RSUDZA in 2017-2019 and had complete data included in this study. Those with other co-infections were excluded, such as hepatitis C virus, HIV, TORCH, and Rubella.

Reactive HBsAg characterizes hepatitis B virus infection. The three primary outcomes of pregnancies were miscarriage (spontaneous abortion), preterm (<37w), or stillbirth (after 20 completed gestational weeks). Utilizing the method age (years) × AST [U/l]/ (platelets [10^9/l] × (ALT [U/l])1/2), The FIB-4 values were automatically computed. The formula [a]AST/ upper limit of normal]/ platelet count [10^9/L][o] × 100 was employed to calculate the APRI values.[14]

An independent t-test analyzed data to examine data with a normal distribution, whereas the Mann-Whitney test was employed to analyze not normally distributed data. This study was registered under number 1171012P and approved by the Ethics Committee for Health Research, Faculty of Medicine, Syiah Kuala University/ Dr. General Hospital. Zainoel Abidin Banda Aceh with number 76/EA/FK-RSUDZA/2019.

Result

In this research, 77 pregnant mothers were found with HBV infection but who have complete data and analyzed as many as 44 people.
The median APRI value was 0.30 (0.1-1.2), while FIB-4 was 0.74 (0.3–1.9). Delivery with live births was found in 42 (95.5%), with term pregnancy 39 (88.6%), vaginal delivery 10 (22.7%), and 14 (31.8%) of pregnancies experienced complications, such as oligohydramnios, HELLP, severe preeclampsia, placenta previa and premature rupture of membranes.

This study found 4.5% of stillbirths with serological markers similar to the live birth group. Age, AST, ALT, APRI, and Fib 4 in the stillbirth group were higher than the live birth group but not statistically different and still in the normal range. Haemoglobin and platelet

Table 1 Baseline Characteristic

Baseline Characteristic	Value
Age (years) (mean±SD)	31.27±5.419
Haemoglobin (g/dl) (mean±SD)	11.41±1.28
Platelet (.10^3/mm^3) (mean±SD)	265. 16±60.60
AST (g/dl) median (min-max)	23 (12–74)
ALT (g/dl) median (min-max)	13.50 (6–76)
APRI, median (min-max)	0.30 (0.1–1.2)
Fib 4, median (min-max)	0.74 (0.3–1.9)
Fetal Maturity n, %	
Preterm	5 (11.4)
Aterm	39 (88.6)
Mode of delivery n, %	
Vaginal Delivery	10 (22.7)
Sectio Caesaria	34 (77.3)
The outcome of pregnancy n, %	
Live birth	42 (95.5)
Stillbirth	2 (4.5)
Obstetric Complication n, %	
Placenta previa	1 (2.3)
HELLP	1 (2.3)
Oligohydramnios	2 (4.5)
Premature rupture of membranes	5 (11.4)
Severe preeclampsia	5 (11.4)
No complication	30 (68.2)

Table 2 Serological Marker Stratified by the Outcome of Pregnancy

	Live Birth (n=42)	Stillbirth (n=2)	P value
Age	31±5.4	37±2.9	0.127
Haemoglobin	11.4±1.3	11.2±1.3	0.855
Platelet	265±60.5	253±86.3	0.775
AST	23 (12–74)	31 (16–46)	0.888
ALT	13.5 (6–76)	18.5 (11–26)	0.821
APRI	0.3 (0.1–1.2)	0.5 (0.2–0.8)	0.682
Fib 4	0.7 (0.3–1.9)	1.2 (0.5–1.8)	0.517

Age, Hb, and platelet use the independent t-test. AST, ALT, APRI, and Fib 4 use the Mann-Whitney test

were lower than in the live birth group.

In this study, 11.4% of cases were found to be preterm. Haemoglobin in the preterm group was lower than in aterm pregnancies. Age, AST, ALT, APRI, and Fib 4 in the preterm group was higher than the aterm group but not statistically different and were still within the normal range.

Discussion

The study of Siakwa et al. observed that babies born from mothers with chronic HBV possess a higher risk for preterm delivery. The general effects of neonatal infections on birth outcomes have been one possible theory that underlies this risk. There has been a significant increase in pro-inflammatory cytokines among infected chronic HBV pregnant women. Several studies report that liver cirrhosis in pregnant women liver cirrhosis may be very harmful.[1]

It is unclear how chronic HBV infection affects pregnancy outcomes. When comparing HBsAg-positive women with controls, one sizable study found no changes in newborn weight, gestational age at delivery, the incidence of prematurity, neonatal jaundice, congenital abnormalities, or perinatal mortality. A relatively recent study, however, found a link between maternal HBV infection (HBsAg positive) and antepartum hemorrhage and gestational diabetes mellitus. There was a hypothesized connection to preterm birth.[15]

Scientific evidence of how chronic hepatitis B infections affect maternal pregnancy outcomes is not widely available. Whether or not the studies on this area of research are yet to be published or the presence of publication bias where studies that found insignificant relationships were not published is still unclear. Another explanation is that Scientists have yet to develop an interest in this area.[1]

Generally, women with chronic hepatitis B infections who are not suffering from advanced liver disease may tolerate pregnancy well. However, some patients show hepatitis signaling, and HBsAg-positive mothers should be monitored closely. Liver biochemical tests were obtained every three months during pregnancy and for six months postpartum. HBV-DNA testing is recommended concurrently or when there is an ALT increase.[15] Chronic hepatitis B without cirrhosis rarely causes problems during pregnancy.[16]

Table 3 Serological Marker Stratified by Fetal Maturity

	Preterm (n=5)	Aterm (n=39)	P value
Age	33.2±4.1	31.03±5.6	0.405
Haemoglobin	10.6±2.5	11.5±1.0	<0.001
Platelet	271±73.9	264±59.8	0.816
AST	35 (16–39)	23 (12–74)	0.159
ALT	18 (9–30)	13 (6–76)	0.616
APRI	0.4 (0.2–0.6)	0.3 (0.1–1.2)	0.502
Fib 4	0.9 (0.4–1.9)	0.7 (0.3–1.8)	0.529

Age, Hb, and platelet use the independent t-test. AST, ALT, APRI, Fib 4 use Mann Whitney test

Most cirrhosis-infected women who conceived achieved a successful pregnancy outcome. ALBI and APRI scores can predict pregnancy outcomes for women with chronic liver illness. Patient treatment in this group was enhanced by preconception counseling from a hepatologist or specialist obstetrician.[9]

In this study, we use a cut-off for significant fibrosis for APRI 1.5 with a specificity of 92% and sensitivity of 39%, and for Fib 4 3,25 with a specificity of 74% and sensitivity of 59%; all of the subjects in this study had no significant liver fibrosis. There is controversy over how hepatitis B affects pregnancy. Only a few studies have shown any adverse outcomes. Pregnancy itself does not affect the hepatitis B infection's natural course.

This research concludes that pregnancy with hepatitis B without fibrosis is not associated with poor pregnancy outcomes. A routine liver function examination is needed in pregnant mothers with hepatitis B virus infection to assess fibrosis. Interprofessional teamwork between internists, gastroenterologists, pediatricians, obstetricians, and midwives is essential in providing a holistic and integrated approach to pregnant women exposed to or who have viral hepatitis to prevent liver fibrosis and good pregnancy outcomes.

This study is paramount because it is the first research in Aceh with a high incidence of HBV infection. This study has limitations because we did not find hepatitis B patients with fibrosis, so we could not assess the pregnancy outcome in fibrosis patients.

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RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n3.3047
Majalah Kedokteran Bandung. 2023;55(3):176–180

Majalah Kedokteran Bandung (MKB)

Received: October 6, 2022
Accepted: February 3, 2023
Available online: September 30, 2023

Comparison of Preemptive Post-Intubation 15 mg/kgBW Paracetamol to 0.35 mg/kgBW Meperidine in Incidence of Post-Anesthetic Shivering

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Abstract

Post anesthesia shivering (PAS) is a repetitive involuntary movement of one or more muscle groups as a result of a decrease in core body temperature. Pharmacological therapy in preventing PAS may include meperidine and paracetamol. This study compared the effectiveness of paracetamol to meperidine in reducing the incidence of post-anesthesia shivering. This study used an experimental randomized double-blind comparative analytic design on patients underwent exploratory laparotomy surgery under general anesthesia at Dr. Hasan Sadikin General Hospital Bandung, Indonesia, from September 2021 to August 2022. Patients with 50 ASA 1-2 physical status were included and divided into two groups. One group received 15 mg/kg group paracetamol and the other received 0.35 mg/kg meperidine. Data on tympanic membrane temperature and hemodynamics before and after induction and after extubating were collected. Furthermore, data on the results of the assessment of the incidence and grade of shivering in each treatment group were also collected. The results of this study showed that there was a decrease in the frequency of PAS in patients receiving intravenous 15 mg/kg paracetamol ($p<0.05$), as well as less side effects in the form of nausea and vomiting ($p<0.05$). The incidence and degree of shivering after general anesthesia using intravenous 15 mg/kg paracetamol was lower compared to the use of 0.35 mg/kg meperidine. In the meperidine group, the decrease in body temperature was lower than in the paracetamol group, while the incidence of nausea and vomiting in the paracetamol group was lower than in the meperidine group. In conclusion, paracetamol reduces the incidence of post-anesthesia shivering better than meperidine.

Keywords: General anesthesia, meperidine, paracetamol, preemptive, shivering

Introduction

Post-anesthesia shivering (PAS) is a repetitive involuntary movement of one or several muscle groups that occurs as a mechanism due to a decrease in core body temperature.[1-4] PAS is a complication that often occurs during the recovery process from anesthesia, with the incidence of shivering after general anesthesia being 40-70%.[1,2,3] General anesthesia will change the process of normal regulation of body temperature by reducing the threshold of the vasoconstrictive response, increasing the threshold of the vasodilation response, sweating, and increasing the inter threshold range from 0,2 °C to 40 °C.[1,3,4] The direct effect of anesthetic drugs occurs in the hypothalamus.[2,3] Shivering poses a risk because of increased oxygen consumption and carbon dioxide production, catecholamines release, cardiac output, tachycardia, hypertension, and intraocular pressure.[1,2,3]

Management of PAS can be done non-pharmacologically by reducing heat loss, such as giving warm infusions, heating pads, or blankets, maintaining the operating room temperature at room temperature, warm blankets, surgical covers, and mattresses filled with warm water.[5,6,7,9] Pharmacological intervention can be done by giving opioids (meperidine, pentazocine, tramadol), α2 agonists (clonidine), magnesium sulfate, ketamine, 5-hydroxytryptamine (5-HT3) receptor antagonists, and non-steroidal anti-inflammatory drugs (NSAIDs).[5,6,7,8] Meperidine was recommended as an agent for preventing PAS, the mechanism of action occurs through the κ receptor and the μ receptor.[9] Meperidine acts as a shivering inhibitor by increasing the

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threshold of sweat response and lowering vasoconstriction.[9] The minimal effective dose of meperidine to prevent PAS is 0.35 mg/kgBW.[9,10,11] The disadvantage of meperidine as a PAS prevention agent is its side effects of nausea, vomiting, and respiratory depression.[10,11]

Paracetamol is an NSAID drug that affects core body temperature through the hypothalamus by affecting prostaglandin synthesis. Paracetamol inhibits cyclooxygenase (COX), which plays a role in the synthesis of prostaglandins in the brain; the substrate is responsible for regulating the set point temperature in the hypothalamus, thereby causing an increase in the threshold for vasodilation and sweating. Paracetamol can be used as antipyretic and postoperative analgesia to reduce opioid consumption. In addition, paracetamol does not have a respiratory depression effect.[10-15] In 2018, research in Iran found that 15 mg/kg BW intravenous paracetamol effectively reduced the incidence of PAS to 9.1% in patients undergoing section cesarean under general anesthesia. [5,6] A study in Japan in 2019 found that 15 mg/kgBW intravenous paracetamol effectively prevented PAS in about 22.2% of patients with gynecologic laparotomy surgery.[7,8]

The study aimed to Compare paracetamol 15 mg/kg BW intravenously with meperidine 0.35 mg/kg BW in terms of the incidence of shivering after general anesthesia.

Methods

In this randomized, double-blinded prospective study, fifty patients underwent laparotomy procedures with general anesthesia in Dr. Hasan Sadikin General Hospital's operating theater from September until December 2021. The criteria for enrollment were patients between 18–60 years old, American Society of Anesthesiologists (ASA) physical status I, II, and surgery duration from one to three hours. Patients who consumed prophylactic drug that affects thermoregulation, history of allergy, elevated liver enzyme, obesity, fever, and or hypothermia were excluded from this study. The study protocol was explained to all patients, and written informed consent was obtained. The Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital Bandung approved the study protocol under the ethical clearance number LB.02.01/X.6.5/325/202. The standard operating room monitoring, including noninvasive blood pressure, electrocardiogram, and pulse oximetry, was performed for all patients. The patients were randomized into two groups based on random allocation. The 1st group received paracetamol 15 mg/kgBW (paracetamol group, n=25), and the 2nd group received meperidine 0.35 mg/kgBW (meperidine group, n=25). Patients were observed for nausea, vomiting, and shivering, and grades were recorded for each patient. The person observing in the postoperative ward was blind to the group allocation. Data recorded age, sex, body mass index (BMI), PAS, body temperature, ASA, room temperature, fluid maintenance, surgery duration, and blood loss volume. Statistical analysis was performed with SPSS 22 for windows and chi-square, and the Fisher test (categorical variables), t-test, and Mann-Whitney (numerical variables) were used. The $p<0.05$ was considered significant.

Results

The statistical analysis results showed no differences in patient characteristics between the two groups in the baseline study. (Table 1) This shows that the research subjects are

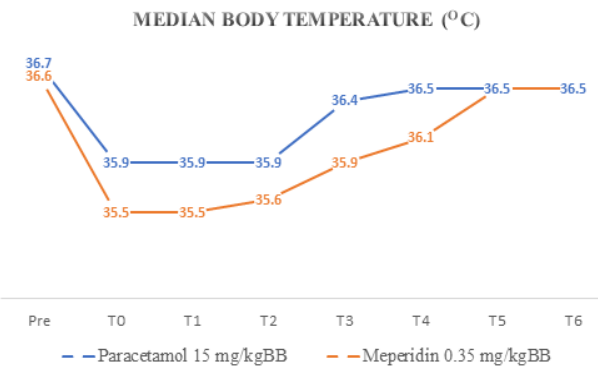

Figure Core Temperature between Two Groups

Table 1 Demographics and Characteristics of Patients

Variables (n=50)	Paracetamol group (P)	Meperidine group (M)	P value
Sex			
Male (%)	4 (16.0)	2 (8.0)	0.667[a]
Female (%)	21 (84.0)	23 (92.0)	
Age (years)			
Mean ± SD	41±8	40±10	0.853[b]
BMI (kg/m^2)			
Median (Range)	22.3 (19.5–30.3)	21.8 (19.6–31.0)	0.382[c]
Duration of surgery (min)			
Median (Range)	135 (120 – 190)	145 (120 – 180)	0.778[c]
Fluid maintenance (mL)			
Median (Range)	1500 (500–1500)	1500 (1000–1500)	0.449[c]
Volume of blood loss (mL)			
Mean ± SD	408±262	464±236	0.428[b]
Room temperature (^{0}C)			
Median (Range)	25.5 (23.5–25.5)	25.5 (23.4–25.5)	0.233[c]
ASA			
I (%)	2 (8.0)	1 (4.0)	1.000[a]
II (%)	23 (92.0)	24 (96.0)	

P value tested with [a] Fisher Exact, [b] t-test, [c] Mann-Whitney test

Table 2 Incidence of PAS

Variables	Paracetamol group (%)	Meperidine group (%)	p-value	RR (95% CI)
Yes (scale 2–4)	2 (8.0)	8 (32.0)	0.034*	0.185 (0.035 – 0.983)
No (scale 0–1)	23 (92.0)	17 (68.0)		

RR=Relative Risk. CI=Confidence Interval. p<0.05 compared with the other groups using the Chi-Square test*

homogeneous and deserve to be compared. The number of PAS is significantly less in the paracetamol group compared to the meperidine group, as shown in Table 2 (p<0.05), with the efficacy of paracetamol being 81.5%.

There were significant differences in postoperative nausea and vomiting or no side effects between the two groups, as shown in Table 3 (p<0.05). Based on Figure, the meperidine group had lower core body temperature post-intubation (p<0.05).

Table 3 Side Effects Between Two Groups After Extubation

Variables	Paracetamol group (%)	Meperidine group (%)	P value
Nausea	1 (4.0)	4 (16.7)	0.015*
Vomiting	0 (0.0)	5 (20.8)	
No side effect	24 (96.0)	15 (62.5)	

*p<0.05 compared with the other groups using the Chi-Square test

Discussion

Postanesthetic shivering is a harmful condition caused by general anesthesia. The side effects can cause hypoxia, pain, and lactic acidosis. Thus, the prevention of shivering is important. This study studied two groups of 25 patients, each candidate for exploratory laparotomy under general anesthesia. The incidence of PAS can be influenced by several factors, including age, BMI, duration of surgery, type of amount of fluid that enters intraoperatively, and operating room temperature.[5,10] In this study. that variable was not significantly different. but this result was the same as other studies.[5,6,7,8] This result may be obtained because the same criteria were set in this study to reduce research bias due to other risk factors. In another study with a larger and more diverse sample size, it was found that the incidence of postanesthetic shivering was influenced by body mass index, duration of surgery, and blood loss.[3]

This study showed that the incidence of PAS was higher in the meperidine group. Based on the result, the researchers concluded that intravenous paracetamol was a more effective prevention drug than meperidine intravenous in preventing PAS due to general anesthesia. This result was similar to the previous study, where paracetamol was more effective than saline infusion. Based on paracetamol's mechanism of action on thermoregulating the core body temperature by affecting the synthesis of Prostaglandin.[5.6] Another study reported that perioperative paracetamol administration can prevent postoperative severe shivering in patients scheduled for gynecological laparotomy. It might be because the drug prevented body temperature from increasing.[7] Until now, no other studies have compared paracetamol with meperidine..

For side effects between the two drugs, results show the meperidine group had more incidence of side effects during the study, and the paracetamol group mostly had no side effects at all. Many studies have reported side effects for other anti-shivering medications, between meperidine and paracetamol. Paracetamol had fewer side effects as an advantage. Meperidine can produce sedation, nausea, vomiting, or respiratory distress.[5] When compared with other anti-shivering drugs. Meperidine has fairly good effectiveness with fewer side effects but still affects respiratory distress.[9.10]

According to this study, patients receiving paracetamol had lower core temperatures than the control group. Still, the two groups had no significant differences, which is consistent with the findings in other studies.[6] This might be that the effects of paracetamol prevented body temperature from increasing. Maintaining core temperature during surgery was important for preventing PAS.[7]

Limitations of this study were the small sample size. Most of the patients were in higher ASA and also limited time for the study. That point can be improved in further studies.

The conclusion of the study is that in addition to maintaining body temperature during surgery. Paracetamol, as a pre-emptive drug in general anesthesia, could help prevent PAS. Besides having fewer side effects, it can also be a postoperative analgesic. More studies should be done on the efficacy of intravenous paracetamol as a preventive agent of PAS.

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RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n3.2777
Majalah Kedokteran Bandung. 2023;55(3):191–196

Majalah Kedokteran Bandung (MKB)

Received: April 14, 2022
Accepted: February 28, 2023
Available online: September 30, 2023

Correlation Between P-Selectin Level and Platelet Aggregation in Cerebral Venous Sinus Thrombosis Patients

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Abstract

One of the causes of cerebral venous sinus thrombosis (CVST) is platelet hyperactivity. Adhesion and secretion are the beginning of platelet activation, which is indicated by a change in the Platelet-selectin (P-selectin) level. The end result of platelet activation is platelet aggregation. However, it is unknown whether the beginning of platelet activation ends with platelet aggregation. This study aimed to discover the correlation between P-selectin level and platelet aggregation in CVST. This study used a cross-sectional escriptive observational correlative approach. Subjects were the CVST outpatients visiting the Department of Neurology Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, from July to September 2021. A total of 49 subjects met the inclusion and exclusion criteria. This study used citrate plasma samples for platelet aggregation and serum for P-selectin assessment. Platelet aggregation were assessed using the light transmission platelet aggregation method while P-selectin was assessed using Enzyme-linked immunosorbent assay (ELISA). Platelet aggregation median was 10.6% (range 0.2–82.4%), which reflected normoaggregation. Platelet hyperaggregation were seen in 9 samples (8.4%). Median of P-selectin was 2.4 ng/mL (range 0.1–10.1 ng/mL) which were normal. High P-selectin level was observed in 16 (32.7%) with 4/16 (25%) experiencing platelet hyperaggregation. Statistical analysis showed a weak negative correlation between P-selectin and platelet aggregation (r=-0.012; p=0.467). In conclusion, no correlation is seen between P-selectin and platelet aggregation, which may be due to the fact that platelets are influenced by many factors that are not examined in this study.

Keywords: Adenosine diphosphate (ADP), cerebral venous sinus thrombosis (CVST), platelet aggregation, platelet-selectin (P-selectin)

Introduction

Cerebral venous sinus thrombosis occurs when a thrombus (blood clot) forms in the brain's venous. Thrombus in CVST forms due to inflammation caused by specific risk factors such as coagulation disorders, autoimmune diseases, acute infections, hormonal disorders, and trauma due to head injuries.[1,2]

Cerebral venous sinus thrombosis cases worldwide are estimated to occur annually in 3-4 people per 1 million population. The patients are more common among young adults rather than elderlies. The disease is more common in females than males (2.9:1).[3]

Impaired blood flow due to venous system blockage will cause increased pressure on brain tissues. Increased pressure on brain tissues will cause brain edema around the venous system blockage area. Capillaries and arterioles will rupture and result in cerebral parenchymal hemorrhage. Bleeding may spread to the nearest subarachnoid space if the pressure increases, causing reduced consciousness or even death.[1,2]

A thrombus or blood clot may form in the vein, artery, heart, or microcirculation. The thrombus may be caused by a deficiency of antithrombin III, proteins C and S, the presence of V Leiden factor, which leads to resistance to protein C activation, direct injury to cerebral sinuses, meningitis, and other prothrombotic disorders such as platelet aggregation. Platelet aggregation may cause approximately 22–25% CVST.[4-6]

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Thrombosis in CVST is generally caused by impaired platelet function and coagulation factors. Abnormalities in platelet activation in CVST cases may be impaired adhesion function, impaired release reactions or secretion, and impaired aggregation function. Due to inflammation, dysfunctional and impaired endothelial structure will cause platelet adhesion, which causes platelets to stick together with collagen in endothelial cells. Then, a reaction will occur to release platelet granules such as ADP, adenosine triphosphate (ATP), norepinephrine, and others to strengthen the aggregation of platelets stuck with collagen. The final process in this thrombosis process is platelet aggregation.[4-6]

Platelet-selectin is a type 1 transmembrane protein on platelet granules and megakaryocyte granules. Activated platelets will experience granular fusion with the endothelial plasma membrane, causing exposure of P-selectin on the surface of the activated platelets. Platelet-selectin also plays a role in mediating interactions between leukocytes and ligand that helps the leukocytes and platelets adhesion process, namely P-selectin glycoprotein ligand-1 (PSGL-1). Platelet-selectin glycoprotein ligand-1 brings leukocyte and platelet cells to attach to the damaged endothelium, which ultimately causes platelet aggregation to occur to plug the wound caused by inflammation. Platelet-selectin causes adhesion and secretion of granules, which in turn causes platelet aggregation.[7,8]

Coagulation factors also contribute to activating platelets. The amount of platelet membrane glycoproteins and the expression of plasma adhesive molecules significantly increased, and many procoagulant platelet factors are released into the blood. Thrombosis can significantly increase the release of P-selectin and other signals of platelet activity. Platelet selection is progressively expressed on the surface of platelets, binding them through the lectin domain to the binding site on adjacent platelets, stabilizing the interaction between bridged platelets and thus enabling the formation of stable, large platelet aggregation. Therefore, the level of platelet aggregation in thrombosis patients increased.[6-8]

It is unknown whether the beginning of platelet activation ends with platelet aggregation. The study aimed to analyze the correlation between the P-selectin level test and platelet aggregation in CVST patients.

Methods

Subjects in this research were CVST outpatients at the Dr. Hasan Sadikin General Hospital Bandung from July to September 2021. Subject inclusion criteria were ≥18 years old, while a history of <200,000/uL platelets became the criteria for exclusion. The research material was 10 ml of patients' plasma for platelet aggregation examination. This blood specimen must be processed within 2 hours, then centrifugated to obtain Platelet Rich Plasma (PRP), and then ADP was added to the PRP. Other materials included 2 ml of patients' serum for P-selectin level examination. Platelet aggregation used ADP 1 μM agonist with light transmission platelet aggregation method. Examination of P-selectin used the enzyme-linked immunosorbent assay (ELISA) method.

This research was a correlative, descriptive observational research with a cross-sectional design. The research data that has been collected was analyzed using Statistical Package for the Social Science (SPSS). Spearman's Rank Correlation test was used for statistical analysis to analyze the correlation between P-selectin level and the result of platelet aggregation examination in CVST patient.

Ethical approval has been received from the Dr. Hasan Sadikin General Hospital Health Research Ethics Committee No. LB.02.02/X.6.5/163/2021. The study has received permission from the Director of Human Resources for Education and Research at Dr Hasan Sadikin General Hospital Bandung No. LB.02.01/X.2.2.1/13394/2021..

Results

A total of 50 research subjects met the inclusion criteria and signed informed consent during the research. One subject was excluded due to a history of 145,000/uL (<200,000/uL) platelet count one month prior. The total number of subjects in this research was 49 patients.

The study used Shapiro-Wilk's normality test on platelet count, aggregation with ADP 1 μM stimulation, and P-selectin data. The normality test result showed abnormal data distribution ($p<0.05$). Thus, non-parametric statistics was used as an analytical approach by presenting data in median and range.

Data on research subject characteristics are presented in Table 1. Data from laboratory examination results of research subjects are presented in Table 2.

Table 1 Research Subject Characteristics Data

Characteristics	Median (Range)	n(%)
Age (years)	48 (22–68)	
Sex		
Male		11 (22.4)
Female		38 (77.6)
Symptom		
Headache		44 (89.8)
Weak on 1 extremity side		8 (16.3)
Seizure		1 (2)
Fainting		2 (4.1)
Others		12 (24.5)
Symptom duration (years)	3 (1–30)	
Risk Factor		
Head injury		5 (10.2)
Autoimmune		8 (16.3)
Hormonal		6 (12.2)
Infection		3 (6.1)
Malignancy		3 (6.1)
Other diseases		10 (20.4)
No risk factor		17 (34.7)
D-Dimer Before Therapy (mg/L)	0.3 (0.2–11.4)	
Abnormal		13 (30.2)
Normal		30 (69.8)
Radiology		
No examination history		3 (6.1)
MRV		23 (46.9)
DSA		9 (18.4)
CT Scan		14 (28.6)
Therapy		
Anticoagulant		45 (91.8)
Antiplatelet		4 (8.2)
Others		30 (61.2)
Duration of therapy (years)	2 (1–8)	

Notes: n: frequency, %: percentage, MRV: magnetic resonance venography; DSA: digital subtraction angiography, CT Scan: computed tomography scan

Table 2 Research Subject Laboratory Characteristic Data

Characteristics	Median (Range)
Platelet (x1000/uL)	352 (204–606)
Aggregation with ADP 1 µM stimulation	10.6 (0.2–82.4)

Notes: n=frequency, %=percentage

Table 3 Correlation between P-selectin Level and ADP 1 µM Platelet Aggregation Test

Research Variable	P-selectin (ng/mL)		
	n	Coefficient r	p-value
ADP 1 µM (%)	49	-0.012	0.467

Notes: Analysis uses Spearman's Rank correlation, *is significant, p<0.05

Normality test result using Shapiro-Wilk's test showed that data was not normally distributed, thus non-parametric Spearman's Rank correlation analysis was used. Table 3 showed the correlation between P-selectin and platelet aggregation based on ADP 1 µM level.

The correlation analysis result showed that there was a very weak negative correlation, and can be neglected, between P-selectin with ADP 1 µM (p>0.05). Figure 1 showed the Bland Altman scatterplot correlation between P-selectin and platelet aggregation test with AD.

The scatterplot above showed no correlation between P-selectin and platelet aggregation test with ADP 1 µM.

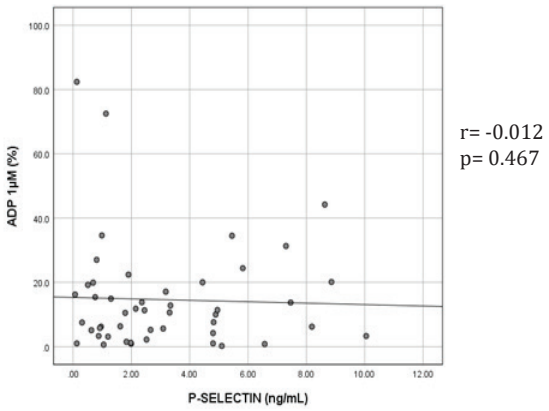

Figure 1 Correlation Scatterplot between P-selectin and Platelet Aggregation ADP 1 µM Test

Discussion

Based on the literature study, this research was the first in Indonesia to analyze the correlation between P-selectin level and the result of platelet aggregation examination in CSVT patients. The median age of research subjects was 48 years old, with a range of 22–68 years old in accordance with research by Ibrahim et al.,[4] who obtained 50% of subjects aged ≥40 years old. Research by Sidhom et al.[9] showed a mean age of 41 years old.This age range is categorized as reproductive age, where estrogen levels in subjects can add to the risk factors for CSVT. Estrogen will decrease by 2% each year entering menopause.

Female comprised the majority of research subjects (77.6%), following the research subjects of Krajickova et al.,[10] where 72.5% was female.Estrogen plays an important role in the occurrence of CVST. Estrogen as a risk factor in females increases coagulation factors and decreases the level of coagulants such as antithrombin. The hormone activates endothelium which interferes with endothelial permeability and causes endothelial damage, resulting in CVST due to thrombus formation. Estrogen also inhibits CYP1A2 and 2C19 and thus it is necessary to increase warfarin dose in CVST patients.[4]

The most frequent symptom in this study was headache (89.9%), with a median onset of 3 years and a range of 1–30 years. The symptom was progressive and persistent and did not improve with some therapy. Headache is caused by venous system blockage, resulting in impaired blood drainage and increased pressure in brain tissues due to a blocked venous system in the dural sinuses. Increased pressure will cause brain edema in the area involved. If the pressure is highly increased, high intracranial pressure will occur and results in the symptom of headache.[10,11]

The majority risk factor was autoimmune diseases (16.3%). This study followed the research of Zhang et al.,[11] who stated that autoimmune diseases caused by an immune response to antigens are an important cause of CVST. Autoimmunity causes inflammatory activation of vascular endothelium and adhesion of leukocyte and platelet components on the endothelium. Autoimmunity leads to hypercoagulability and easily damaged endothelium, which results in thrombosis.

The range of treatment (1–8 years) made variations in P-selectin results. The administration of drugs mainly consisted of anticoagulants (91.8%) and antiplatelets (8.2%). Aside from anticoagulants or antiplatelets, this research's subjects comprise several other drugs, such as steroids for autoimmune diseases, dyslipidemia, hypertension drugs, etc. All subjects have been given therapy; thus, their treatment history was not a confounding factor but became findings in this research.

Anticoagulants are necessary for CVST to prevent thrombus growth, facilitate recanalization, and prevent the risk of DVT. The subjects consumed Warfarin, an oral anticoagulant that is commonly used. The drug affects vitamin K synthesis, which contributes to blood clotting, resulting in the depletion of factors II, VII, IX, and X. Warfarin acts in the liver by inhibiting vitamin K carboxylation from its precursor protein. Due to the half-time of each blood clotting factor, depletion of factor VII will prolong the prothrombin time. As mentioned earlier, the anti-thrombotic effect only reaches its peak after the depletion of the four factors. The anticoagulant effect of warfarin will require several days.[12]

Antiplatelet drugs can affect the result of platelet aggregation examination, but in this research the subjects still consumed the drug due to the difficulty in obtaining research subjects. Aspirin works by irreversibly acetylating Ser529 in cyclooxygenase (COX-1), inhibiting the formation of thromboxane A2 (TXA2). Thromboxane A2 is a potent platelet aggregation that may strengthen platelet aggregation. Platelet aggregation inhibition due to aspirin consumption is irreversible because it occurs according to the age of platelets. Other antiplatelets, such as Clopidogrel, act on the adenosine receptor as the antagonist. Clopidogrel irreversibly inhibits the P2Y12 receptor, one of the adenosine receptors on platelets. Clopidogrel inhibits ADP-induced platelet aggregation.[13]

Endothelial dysfunction due to inflammation in CVST will cause the occurrence of platelet activation as well as an impaired coagulation system. Activation of platelets can be demonstrated by an increased level of P-selectin as well as platelet aggregation. Platelet aggregation will cause fibrin clots. Fibrin clot degradation will occur to maintain fibrinolytic system balance. Among fragments produced during fibrin clot degradation is D-dimer. Clinicians use D-dimer as a diagnostic parameter in CVST management.[14]

The median D-dimer history was 0.3 mg/L with a range of 0,2–11,4 mg/L, and 30,2% of subjects had increased D-dimer. A well-

designed prospective study on 343 patients showed that D-dimer levels decreased over time since symptom onset. In contrast, patients with sub-acute or chronic symptoms produced normal D-dimer results. Anatomic expansion of thrombosis sinus location in patients with extensive obstruction may also lead to a false low D-dimer result.[14]

Hyperaggregation due to ADP 1 µM stimulation was present in 18.4% of subjects, which is to the etiology of CVST that 22–25% of thrombosis is caused by platelet hyperaggregation in cerebral vessels.4-6 Platelet hyperactivity in thrombosis patients causes hyperaggregation to quickly occur when administering a low dose of an agonist such as ADP 1 µM.[15] Therefore, this research measured platelet aggregation with ADP 1 µM stimulation.

P-selectin level, according to the insert kit, is divided into 3 categories in which 0.47–0.53 ng/mL make up the low category, 0.97–1.03 ng/mL as the normal category, and 3.50–3.90 ng/mL as high category.[16] This research showed high P-selectin levels in 16 subjects, but most subjects showed normal P-selectin levels in 22 subjects and low levels in 11 subjects.

Platelet aggregation occurs due to the hyperactivity of platelets caused by active platelets, which is demonstrated by the increased level of P-selectin.[17] The lack of correlation between P-selectin level and platelet aggregation results in research subjects may be caused by many factors.

Many factors influence platelet aggregation. After platelets' initial contact with the subendothelial extracellular matrix, which is mediated by Von Willebrand Factor (VWF) and Glycoprotein Ib (GPIb), platelet adhesion occurs mediated by integrin and multiple cellular activations such as P-selectin. Then, platelet activation occurs by collagen, secretion of mediators such as ADP, thromboxane A2, or thrombin, and platelet shape changes. Mediators are still necessary to recruit platelets into the platelet plug to result in platelet aggregation in CVST patients.[18]

Not all platelets will be activated with the presence of a stimulus. Research by Wild et al. stated that according to standards, there are only 36% of activated platelets in platelet concentrate storage.[19] Based on this, there are variations in platelet activity, namely increased P-selectin level and platelet hyperaggregation due to activated platelets, as well as P-selectin level that does not increase and platelet normoaggregation or hypoaggregation because platelets are not activated. This causes variety in P-selectin level and platelet aggregation.[15]

ADP 1 µM agonist is reversible; thus, there is a possibility that a platelet plug will not form. In a low ADP level (1 µM), the light inductor will slightly increase and decrease due to the reversible aggregation. Platelets quickly experience deaggregation.[20] GPIIb/IIIa complex activation results in fibrinogen binding and platelet aggregation that may occur with a lower agonist level than required to produce degranulation. Stimulated platelets do not always lead to a fully irreversible aggregation. Meanwhile, P-selectin expression is a reversible process. Thus, P-selectin can be released from platelets during this phase and increases in subjects.[7,8]

Platelet activity includes increased P-selectin level after 1 hour and a maximum of 40 hours; according to research by Stokol et al.[21] Aggregated platelets can only survive up to 10 days throughout the platelet's life cycle.[15,20] Research by Lukasik et al.[18] showed that P-selectin may significantly decrease after a stroke. This research showed that P-selectin increases in acute and subacute stroke but decreases to normal in the 90th day (3 months). Research by Van Golen et al.[20] showed that platelet activity did not increase after liver ischemia, and thus, there was no increase in P-selectin level.

There are multifactorial risk factors underlying CVST such as autoimmune, hormonal therapy, head injury, infection, malignancy, and other diseases. These risk factors can also affect the result of P-selectin level examination using the ELISA method.[22]

The research concludes that no correlation exists between P-selectin level and platelet aggregation due to the many factors influencing the different results between the two variables. Further research is recommended to determine the relationship between P-selectin level and platelet aggregation test in CVST patients in subjects who have not received anticoagulant and antiplatelet therapies or with P-selectin level examination using other methods such as flow cytometry.

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RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n3.3149
Majalah Kedokteran Bandung. 2023;55(3):149–153

Majalah Kedokteran Bandung (MKB)

Received: December 28, 2022
Accepted: May 22, 2023
Available online: September 30, 2023

Differences in Neutrophil Lymphocyte Ratio (NLR) between Sepsis and Septic Shock Patients in a Tertiary Hospital in Indonesia

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Abstract

Sepsis is a severe medical condition characterized by organ dysfunctions resulting from the body's response to an infection. Septic shock is a complication of sepsis that involves circulatory, cellular, and metabolic disturbances. The body's response to infection is typically marked by an increase in neutrophils and a decrease in lymphocytes, leading to increased Neutrophil Lymphocyte Ratio (NLR). This study aimed to investigate the differences in NLR between sepsis and septic shock patients treated at Dr. H. Abdul Moeloek Provincial Hospital, Lampung, Indonesia. This study utilized a comparative analytic study approach with a cross sectional design. A total of 96 sepsis patients who met the inclusion and exclusion criteria treated during the period January 2018–December 2020 at Dr. H. Abdul Moeloek Provincial Hospital, Lampung, Indonesia, were included in this study. The study found that most of sepsis patients showed an increase in neutrophils (89.58%), a decrease in lymphocytes (94.79%), and elevated NLR values (87.5%). The mean NLR value of septic shock patients (20.10±5.89) was significantly higher than that of sepsis patients (18.01±1.35) (p=0.025). It indicates that the NLR value increases significantly in patients who develop septic shock compared to those with sepsis alone. The findings suggest that NLR could be a potential biomarker for septic shock diagnosis. However, the effectiveness of NLR in predicting the severity of sepsis and determining the risk of mortality in these patients still needs to be examined by conducting studies that involve a larger sample size.

Keywords: Neutrofil Lymphocyte Ratio (NLR), sepsis, septic shock

Introduction

Sepsis is among the most common health issues among populations experiencing severe clinical infections. It is characterized by typical inflammatory responses, such as vasodilation, leukocyte accumulation, and increased microvascular permeability in tissues far from the infection site's source.[1] In 2016, the International Society of Critical Care Medicine (SCCM) and the European Society of Intensive Care Medicine (ESICM) suggested the term sepsis-3 to describe the sepsis condition. According to that, sepsis is defined as a life-threatening organ dysfunction caused by a body's response to an infectious disorder. Sepsis shock is a severe form of sepsis accompanied by hypotension despite adequate fluid resuscitation. It requires vasopressors to maintain a mean arterial pressure of ≥65 mmHg or a lactate concentration in the blood of >2 mmol/L (>18 mg/dL).[2,3]

A study conducted in 2009 across 16 countries in Asia, including Indonesia, discovered that the incidence of severe sepsis and septic shock in intensive care units (ICUs) was 10.9%, with a mortality rate of 44%. Similarly, a study at RSCM Jakarta in 2012 found that out of 84 intensive care cases, 23 were diagnosed with severe sepsis and septic shock, and the mortality rate reached 47.8%.[4]

Leukocytes play a crucial role in the systemic inflammatory response, particularly in severe infections, trauma, and shock.[5] During systemic inflammation, the number of neutrophils tends to increase, while the number of lymphocytes tends to decrease, resulting in an increased of neutrophil lymphocyte ratio (NLR). The neutrophil lymphocyte ratio (NLR) is determined by comparing the absolute neutrophil count to the absolute lymphocyte count, which can be obtained by examining the leukocyte count in the blood sample.[6,7] Sepsis may cause a rise in the NLR value due to increased lymphocyte cell apoptosis. In addition, septic shock is known to cause a considerable decrease in lymphocyte

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count, leading to a significant increase in the NLR value.

Previous studies have demonstrated that the NLR value can serve as a valuable prognostic marker for sepsis patients in the ICU. However, research has yet to be conducted on NLR values in sepsis patients in Lampung, specifically at Dr. H. Abdul Moeloek Hospital. This is a significant gap, given that the NLR value has the potential to support early, affordable, and rapid diagnosis of shock sepsis. Thus, this study aims to investigate the differences in NLR values between patients with sepsis and septic shock at RSUD Dr. H. Abdul Moeloek Lampung.

Methods

This was a comparative analytic study with a cross sectional design. This study was conducted at Dr. H. Abdul Moeloek Hospital Lampung between December 2020 to January 2021, using medical record data from January 2018 to December 2020.

The inclusion criteria for this study were adult patients (≥18 years old) diagnosed with sepsis and septic shock, with complete blood test results recorded in their medical records. They agreed to participate by signing an informed consent form. Sepsis was diagnosed based on a SOFA score ≥2. Septic shock was diagnosed based on SOFA score ≥2 and lactate levels ≥2 mmol/L (>18 mg/dL), as well as a requirement for vasopressors to maintain mean arterial pressure of ≥65 mmHg. The exclusion criteria were pregnant females, patients with immunocompromised conditions such as liver failure, autoimmune disease, hematological cancer, and AIDS, and patients with severe illnesses such as metastatic cancer, stroke, coma, and advanced heart failure.

This study uses consecutive sampling methods, meaning that all patients who met the inclusion and exclusion criteria were enrolled. Ninety-six patients who met the criteria were included in the study and were divided into two groups based on disease severity: the sepsis group, consisting of 77 patients, and the septic shock group, consisting of 19 patients. Personal medical history, age, clinical signs, symptoms, and laboratory blood test results were collected from all subjects from their medical records. All data were analyzed using SPSS for Windows (version 24.0, Chicago, Illinois, USA). Quantitative data were expressed as mean with standard deviation, while qualitative data were expressed as frequency and percentage. The difference in NLR values between sepsis and septic shock patients was analyzed using an independent t-test with a significant level (α) of 5%.

This research has undergone ethical review and obtained an ethical clearance letter from the Health Research Ethics Committee, Faculty of Medicine, University of Lampung, with letter number 30906/UN26.18/PP.05.02.00/2020.

Table 1 General Characteristics of Subject

Variables	Frequency	Percentage (%)
Gender		
Male	53	55.21
Female	43	44.79
Age (years±SD)	54.68±15.67	
18–25	4	4.17
26–35	8	8.33
36–45	11	11.46
46–55	23	23.96
56–65	24	25.00
>65 years	26	27.08
Neutrophil		
Neutropenia	0	0
Normal	10	10,42
Neutrophilia	86	89,58
Lymphocyte		
Lymphopenia	91	94,79
Normal	5	5,21
Lymphocytosis	0	0
NLR		
<5	12	12.5
>5	84	87.5

Results

This study includes 96 patients with sepsis diagnosis according to ICD X: A41.9. Most of the subjects in this study were male (55.21%) with an average age of 54.68±15.67 years. The age distribution was dominated by individuals over 45 years old, and the older the age group, the higher the number of subjects. As predicted earlier, most of the subjects showed an increase in the number of neutrophils (89.58%), a decrease in lymphocytes (94.79%), and an increase in NLR value (87.5%) (Table 1).

Table 2 Difference of NLR Value Between Sepsis and Septic Shock

Variables	Total (n=96)	Sepsis (n=77)	Septic shock (n=19)	p
Neutrophils (%)	88 (52–97)	84 (75–96)	90 (52–97)	0.183
Lymphocyte (%)	6 (1–42)	10 (1–23)	6 (2–42)	0.247
NLR	18.45±1.51	18.106±1.35	20.10±2.89	0.025*

Note: * showed a significant difference between sepsis and septic shock at α=5%

The neutrophil count showed no significant difference between sepsis and septic shock. Similarly to the neutrophil count, lymphocyte count also did not show a significant difference between these two groups. The study also found that most patients had an NLR value higher than the normal NLR range, averaging 18.45±1.51. The NLR values were significantly higher in patients with septic shock (20.10±5.89) compared to those in the sepsis group (18.01±1.35) (p=0.025) (Table 2).

Discussion

Leukocyte count has long been used as a diagnostic parameter for sepsis or septic shock. However, not all septic patients exhibit abnormalities in their leukocyte count, as some may have normal leukocyte counts.[8-10] In recent years, researchers have sought new variables that might be more accurate in diagnosing sepsis. One such promising variable is the LNR value.

This study involved 96 subjects, most of whom were sepsis patients (80.21%), while the remaining had septic shock (19.79%). These findings align with Huang et al.'s study, which found that the number of sepsis patients was higher than that of septic shock patients (23 vs. 22, respectively).[17] Another study by Nainggolan et al. reported a higher prevalence of sepsis (59.7%) than septic shock (40.3%).[18] As Martin notes, intensive care services are typically required when shock sepsis accounts for over 50% of patients.[19]

The study also found that sepsis was more prevalent among older individuals (Table 1), consistent with previous research such as the study by Starr and Saiyo.[13,14] One possible explanation for this discrepancy is the influence of estrogen, a hormone known to have a protective effect against infection, sepsis, and trauma. Estrogen is involved in increasing various anti-inflammatory cytokines, including IL-4 and IL-10, which can indirectly stimulated antibody production.

The study also found that sepsis was more prevalent among older individuals (Table 1), which is consistent with previous research such as the study by Starr and Saiyo. In the United States, over half of sepsis cases are diagnosed in patients aged 65 years and above, with higher mortality rates than younger patients. Sepsis poses a more significant threat to geriatric patients because aging weakens their immune systems, making them more susceptible to infections and sepsis (Tamba Doang). Both innate and adaptive immune responses decline with age, which can contribute to increased infections among the elderly. The reduction in adaptive immune responses is associated with decreased immune function and a decline in the number of cells involved in the body's immune system. Specifically, the number of B cells and generation of T cells decreases with aging, reducing response to new pathogens. However, the ability to mount an efficient response to pathogens remains intact.[14]

Most of the samples in this study had neutrophilia and lymphopenia (Table 1). This study also revealed that the neutrophil count of the subject was overall elevated, while the lymphocyte count was decreased, but similar in sepsis and septic shock (Table 2). Neutrophilia is a characteristic feature of sepsis caused by bacterial invasion, which triggers the mobilization and rapid migration of immune cells (neutrophils) from the bone marrow to the circulation, resulting in a shift to the left.[16] Conversely, lymphopenia is often observed in severe systemic inflammation such as sepsis, involving marginalization and redistribution of lymphocytes in the lymphatic system, leading to accelerated of apoptosis. The apoptosis process is initiated when macrophages release proapoptotic agents such as TNF-α, Nitrite oxide (NO), and glucocorticoids, which suppress lymphocyte production.[13,17]

This study also found an increase in NLR values, with significantly higher values in

patients with septic shock than those with sepsis alone (Table 2). This finding suggests that NLR may have potential value in assessing the severity of sepsis. These results are consistent with a study by Zahorec, which investigated the relationship between lymphocyte and neutrophil counts during systemic inflammatory responses and found that the ratio of lymphocyte to neutrophil counts is a reliable, fast, and simple method for evaluating inflammatory stress.[20] This is also supported by research conducted at Dr. Hasan Sadikin General Hospital Bandung in 2013, which showed a relationship between NLR and SOFA scores in ICU patients, where systemic inflammatory conditions such as sepsis were marked by an increase in NLR and organ failure was marked by an increase in SOFA scores.[21]

This study has some limitations, as it relied on secondary data from medical records, which may have been affected by the quality of documentation by healthcare providers. As a result, the researchers needed help understanding the variables studied comprehensively. There were also limitations in collecting medical record data, which often needed to be completed, especially regarding the history and physical examination record.

In conclusion, NLR is a simple parameter that can be easily measured through a complete blood count, a standard and required test for all hospitalized patients. The NLR values in shock septic were significantly higher than those with sepsis alone. Therefore, it has the potential to be widely utilized in assessing the severity of sepsis or septic shock. However, more extensive studies are necessary to confirm the effectiveness of NLR in predicting the severity of sepsis and determining the risk of mortality in these patients by involving a larger sample size.

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RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n3.3112
Majalah Kedokteran Bandung. 2023;55(3):186–190

Majalah Kedokteran Bandung (MKB)

Received: November 27, 2022
Accepted: May 22, 2023
Available online: September 30, 2023

Effect of Community Education on Community Knowledge of Premature Rupture of Membranes

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Abstract

Premature rupture of membranes (PROM) is still a health problem with a reasonably high incidence among pregnant women in Indonesia. The PROM is defined as the rupture of membranes before signs of labor are observed. Better knowledge on the causes, signs, symptoms, and complications of PROM is believed to help in reducing maternal and infant mortality caused by PROM. This was a cross-sectional, analytic observational study conducted in Cipacing Village, Sumedang, West Java, Indonesia during the period of June–July 2022. Community education was held to give better knowledge about PROM for women with an obstetric and gynecology specialist and fetomaternal consultant as the resource person. Total sampling was obtained from 62 women. Data were collection using pre-test and post-tests before and after the education session. The mean pre-test score was 6.48, while the mean post-test score was 7.96. A dependent T-test was used to determine the relationship between scores before and after the community education with a p-value of 0.0001. There was a significant increase in knowledge about PROM after the education session compared to before the session. This means community education is effective and impactful to increase the level of knowledge about PROM among women.

Keywords: Community education, group education, knowledge, PROM

Introduction

Maternal mortality rate (MMR) is one indicator used to see women's health status in an area. This indicator can assess maternal health programs and the degree of public health because of their sensitivity to improving health services in terms of accessibility and quality. The decline in MMR in Indonesia occurred from 1991 to 2007, from 390 to 228. However, the 2012 Indonesian Demographic and Health Survey (SDKI) showed a significant increase in MMR, 359 maternal deaths per 100,000 live births. MMR again showed a decline to 305 maternal deaths per 100,000 live births based on the 2015 Intercensus Population Survey (SUPAS) result.[1]

According to the World Health Organization (WHO), around 830 women worldwide die from complications during pregnancy and childbirth. Approximately 99% of all maternal deaths occur in developing countries. The number of maternal deaths worldwide in 2018 was 303,000 people, and the number of maternal deaths due to pregnancy and childbirth complications worldwide was 216. One of the causes is caused by premature rupture of membranes.[2,3]

Premature rupture of membranes (PROM) is still a health problem in Indonesia, with a reasonably high incidence and mortality rate; premature rupture of membranes is close to 10% of all deliveries. At less than 34 weeks of gestation, it is around 4%. Meanwhile, according to Fadli, the incidence of PROM in Indonesia was 35.70% -55.30% of 17,665 births.[1] Premature rupture of membranes (PROM) is defined as rupture of the membranes before signs of labor. This can occur in pregnancy at term or in preterm pregnancy. PROM is the most significant cause of early labor with various consequences.[4,5] PROM is a state of rupture of the membranes before delivery. If PROM occurs before 37 weeks of gestation, it is referred to as premature rupture of membranes in preterm pregnancy or Preterm Premature Rupture of Membranes (PPROM).[6]

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Better knowledge about PROM's causes, signs, symptoms, and complications will go one step further in reducing maternal and infant mortality.[7]

This study aimed to determine the relationship between the level of knowledge before and after community education in terms of pre-test and post-test scores.

Methods

This research was a cross-sectional, analytic observational study conducted in Cipacing Village, Sumedang, West Java, from June to July 2022. This study obtained Ethical approval from The Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital Bandung with the number LB.02.01/X.6.5/448/2022. The research subjects were all women who attended the community education about PROM at the Cipacing Village Office on July 18th, 2022. All of them had various professions and backgrounds in the productive age range of 20-36 years old. The event was held in a sports hall within the village office. The media used were interactive PowerPoint presentations, flyers, and posters. The subjects were first asked to do the pre-test before listening to the PROM explanationThey can then ask questions to the speaker regarding the topics. The study obtained a total sampling of 62 women. The inclusion criteria were that subjects completed the pre-test, attended community education, and completed the post-test. This study's exclusion criteria were subjects who did not meet the test. The scores were then categorized as high, adequate, and low levels of knowledge about PROM.

This study analyzed the research data with Statistical Package for the Social Sciences (SPSS) version 26 for Mac, where the data were divided into univariate and bivariate data. Univariate data showed the respondents' distribution based on age and level of knowledge. Bivariate data showed the respondents' distribution based on the relationship between the level of knowledge before and after community education in terms of pre-test and post-test. The Shapiro-Wilk test was used to measure the data normality. The dependent T-test and Fisher exact test were used to analyze the bivariate data. The data were presented in tables.

Table 1 Subjects Characteristics

Variables	Frequency	
	n=62	%
Age (years)		
<20	13	21.0
20–35	24	38.7
>35	25	40.3
Mean±SD	33.1±14.4	
Pre-test Score		
Mean±SD	6.48±1.45	
Post-test Score		
Mean±SD	7.96±0.92	
Level of knowledge		
Low	14	22.6
Adequate	29	46.8
High	19	30.6

Results

This research was a cross-sectional, analytic observational study conducted in Cipacing Village, Sumedang, West Java. The research subjects were all women who attended the community education about PROM at the Cipacing Village Office. The study obtained a total sampling of sixty-two women. The inclusion criteria were that subjects completed the pre-test, attended the massive counseling, and completed the post-test. This study's exclusion criteria were subjects who did not meet the test.

Table 1 presents subjects' characteristics based on age, pre-test score, post-test score, and level of knowledge. The mean pre-test score is 6.48, while the mean post-test score is 7.96. Table 1 shows that most respondents have adequate knowledge about the premature rupture of membranes (46.8%). The test scores determine the level of knowledge. A value below 55% is declared low, between 55% and 76% is declared adequate, and over 76% is declared high.

The Shapiro-Wilk test was done to see the normality of the data, and the result showed a normal distribution in age, pre-test, and post-test scores. Therefore, a Fisher exact test was used to determine the relationship between age and level of knowledge. Table 2 shows that age and level of knowledge have no significant relationship. This means age does not affect the level of knowledge about PROM.

The effect of massive counseling in this study can be seen from the difference in test scores before and after implementing the massive counseling intervention. A dependent T-test was used to determine the relationship between

Table 2 Relationship between Age and Level of Knowledge

Variables	Level of knowledge						p value
	Low		Adequate		High		
	n	%	n	%	n	%	
Age (years)							
<20	4	6.45	7	11.29	2	3.22	0.415
20–35	6	9.67	10	16.12	6	9.67	
>35	4	6.45	12	19.35	11	17.74	

Table 3 Pre-Test and Post-Test Score Distribution

		p value
Pre-test Score		
Median	7.00	
Min-Max	1.00-9.00	
Mean±SD	6.48±1.45	0.0001*
Post-test Score		
Median	8.00	
Min-Max	6.00-10.00	
Mean±SD	7.96±0.92	

*p value significant

scores before and after the massive counseling with p-value = 0.0001. Table 3 shows a significant difference between the pre-test and post-test scores. This means the massive counseling is effective and impactful towards the level of knowledge about PROM.

At the time of the post-test, most subjects experienced an increase in scores, namely as many as 49 people (79%). Subjects whose scores were fixed were 10 people (16.1%), and those whose scores were decreased were 3 people (4.8%).

The N-gain score is then carried out to determine the effectiveness of a method or treatment. The results obtained are 0.36, which is in the moderate category, so it can be interpreted that the massive counseling method is quite effective (0.3≤g≤0.7) in increasing knowledge about PROM.

Table 4 Distribution f Score Elevation

Score	n	%
Increase	49	79
Stagnant	10	16.1
Decrease	3	4.8

Discussion

Knowledge is an abstract concept without any reference to the tangible world.[8] However, knowledge could be interpreted as human sensing or knowing about an object through the five senses. Sensing occurs through the five human senses, namely the senses of sight, hearing, smell, taste, and touch.[9] According to Dale, the capture of knowledge imparted through the sense of sight is 75-87%, through the sense of hearing is 13%, and 12% from other senses. The more senses are involved in capturing messages, the easier the message can be received by educational targets.[10] In this study, community education can obtain subject knowledge through the senses of sight and hearing using interactive presentation media. Other studies using the same media show significant differences in knowledge scores on subjects before and after being given the education.[11] There was a significant increase in dental and oral health knowledge scores before and after being given counseling with media presentations.[12] Poster media is also effective as an educational medium because it helps stimulate the sense of sight. In addition, the visual aspects of poster images also make it easier to receive information or educational materials.[9]

There is a significant difference in average PROM knowledge before and after the intervention. The existence of respondents who did not experience an increase in knowledge or even experienced a decrease in score could be caused by several things. The decrease in the knowledge score can be caused by the need for more concentration of respondents in participating in the research process, filling out the pre-test and post-test questionnaires, and participating in community education. This may be because the education was carried out at the Village Office Sports Hall, so the voices tended to resonate. Conditions of the event that could be more conducive can reduce the focus and

enthusiasm of respondents to discuss and listen to educational material. To assess the effect of education, knowing how significant the effect is on knowledge is necessary.

Educational effectiveness is the success of education as measured by the increase in the value of the respondent's knowledge after education. Effectiveness is used to assess the level of program success and target achievement and compare the effectiveness of several programs.[13,14]

In this study, what is meant by results in effectiveness is the number of respondents who experience an increase in knowledge. The number of respondents who experienced an increase in knowledge was 49 (79%). Based on the calculation of the N-gain score formula, the result is 0.36, which is in the moderate category, so it can be interpreted that the community education method is quite effective ($0.3 \leq g \leq 0.7$) in increasing knowledge about PROM.

There is a significant difference in knowledge about PROM between before and after counseling with p-value=0.0001 with an N-gain score of 0.36. It is better to conduct research with a quasi-experimental design using a control group for future studies.

In conclusion, community education is effective and impactful toward the level of knowledge about PROM.

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RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n3.3155
Majalah Kedokteran Bandung. 2023;55(3):154–160

Majalah Kedokteran Bandung (MKB)

Received: January 5, 2023
Accepted: May 30, 2023
Available online: September 30, 2023

Hepatoprotective Potentials of Dates Extract (*Phoenix dactylifera*) in Acetaminophen-Induced Mice

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Abstract

Dates (Phoenix dactylifera) are considered as a well-known fruit consumed by many people in various countries. This study aimed to examine potential effects of dates as a hepatoprotective agent in mice. This study was conducted at the Iratco Group's eLRosa Laboratory Research Facility, Indonesia, starting from June 2022 to July 2022. Mice from ddY strain were randomly divided into 5 groups (n=5 per group) of positive and negative control groups, and treatment groups 1, 2, and 3. The negative control group as the normal baseline did not receive acetaminophen and date extract. In treatment groups, 30 µL/30 grBW, 60 µL/30 grBW, and 100 µL/30 grBW extract was given per oral to Treatment Group 1, Group 2, and Group 3, respectively for 20 days. On day 21, all treatment groups were induced with 300mg/kgBW acetaminophen for 3 days via the intraperitoneal route. Blood tests were performed on day 24 to measure the serum transaminase level as the parameter of liver damage. The lowest level of transaminase serum was found in group 3 with the highest volume of dates extract, which was 100 µL, followed by group 2 (60 µL), and group 1 (30 µL). There was a significant difference between the positive control group and treatment groups with no significant difference was seen between negative and the treatment groups. This study concludes that dates extract has the potential of being a hepatoprotective agent.

Keywords: Acetaminophen, dates, hepatoprotection, mice

Introduction

The liver is an organ with complex and diverse roles for each individual. In particular, the function of the liver is to filter blood from the intestines through the venous port, then store it and convert food materials received by the venous port. The liver also protects other organs in the body, especially the brain, against toxic substances that are absorbed through the intestine (detoxification), such as ammonia. Kupffer cells are in the liver and work with phagocytizing bacteria and proteins that enter the venous port system through the intestinal wall.[1] Liver damage will cause disturbance to liver functions, and ultimately, resulting in health and body homeostasis. Liver damage or liver disease is a life-threatening disease. Liver damage causes two million deaths annually, with a percentage of one million deaths due to cirrhosis and one million deaths due to viral hepatitis and hepatocellular carcinoma.[2]

One of the most common causes of liver damage is long-term exposure to hepatotoxic drugs (Drug-induced liver injury) and chemicals. This exposure will cause changes in liver cells, especially in hepatocytes. Such as fat degeneration and necrosis can reduce the ability of cell regeneration, causing permanent damage to cell death.[3] One of the drugs classified as a drug that can induce liver injury is acetaminophen. Acetaminophen belongs to a class of analgesic and antipyretic drugs. Acetaminophen is safe and effective, but excessive use can cause liver damage. Liver damage due to excessive use of acetaminophen is caused by the metabolism

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of acetaminophen in the form of N-acetyl-p-benzoquinone (NAPQI), which cannot be completely neutralized by hepatic glutathione. NAPQI will bind to liver cell proteins and cause liver damage.[4]

Liver damage can be measured through the activity of transaminase serum. Two well-known transaminase serums are serum glutamate pyruvate transferase (SGPT) and serum glutamate oxaloacetate transferase (SGOT). SGPT can be found in liver cells, heart, muscle, and kidneys. The greatest portion of SGPT can be found in the liver cytoplasm. On the other hand, SGOT can be found inside the heart cells, liver cells, muscles, kidneys, brain, pancreas, lymph, and lungs. The highest level of SGOT can be found inside the heart cells. The change of permeability causes the increase of SGPT and SGOT or damages the cell wall. Thus, SGPT and SGOT can be used as markers of liver cell damage.[5]

Treatment of liver damage can be done with chemical drugs. Alas, the development of liver damage drugs is still not well developed. It has caused several medication-related problems (MRPs), which happen when the usage of therapeutic drugs does not result in any positive effects. However, on the contrary, it results in adverse effect.[6] Based on that fact, the exploration of the hepatoprotective effect of herbal medication and the composition of active ingredients in herbs shall be done. Herbal medicine does not tend to cause MRPs or any side effects, is safe for long-term usage, can be obtained at low prices, and contains a lot of substances with phytoestrogenic effects, antioxidants, and nutrition. [7]

Some herbal plants have been proven to have a hepatoprotective effect; one is bean sprouts. Based on research conducted by Liu et al.,[9] bean sprouts have been proven to have a hepatoprotective effect on the liver, even on the livers that are already damaged in mice.[8] This is due to the flavonoid in bean sprouts. Tumeric also has the potential as a hepatoprotective agent besides bean sprouts. Due to its potent antioxidant effect and anti-inflammation, turmeric can act as a hepatoprotective agent. Another herbal plant that has hepatoprotective potential is dates. Dates (*Phoenix dactylifera*) are a large commodity and an essential crop in hot, arid regions such as Saudi Arabia and Egypt. In these countries, dates are commonly used as medicine and cosmetics and consumed by humans and animals.[10] Apart from these countries, dates are also famous in Indonesia because of their sweet taste, many benefits, and the fact that they can easily be obtained. Based on the result of phytochemical analysis, it was reported that dates contain alkaloids, steroids, flavonoids, vitamins, and tannin. Flavonoids are known to have membrane stabilizing, hepatoprotective, and antioxidant activity.[11] Dates also contain vitamin C and bioactive components such as carotenoid, sterol, tannin, isoflavonoid, flavonoid, and phenolic acid. These components have an inhibitory effect against oxidative damage.[12,13] Based on research by Alothaid, date extract was proven to improve antioxidant activity in the liver by stimulating the production of CAT, GPx, SOD, and GSH.[14]

This research is conducted to observe the potential hepatoprotective effects of dates on transaminase serum in mice. This research is expected to provide an overview of the potential hepatoprotective effect of dates. In addition, the result of this study is also expected to be used as a basis of consideration and a reference for future research on using dates as hepatoprotective agents.

Methods

The research was conducted at the IRatCo Group's eLRosa Laboratory Research Facility. The research was held from June 2022 to July 2022. The research was carried out from June 2022 to July 2022. The research procedure of using mice has previously met the ethical rules from the Health Research Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences IPB University with a certificate of ethical clearance Number. 060/KEH/SKE/XII/2021. Mice that were used were mice with ddY *Strain*, aged eight weeks, all female, and were not pregnant. Mice were grouped into five groups, with each group containing 5 mice. The number of experimental animals used was calculated with the Federer formula (1997). The calculation of the experimental animal is as follows.

Based on the calculation with the Federer formula, it was concluded that each treatment needed at least 5 mice. Acclimatization of experimental animals is a step that must be done before using them in the research, with consideration of possible differences in the environment from the place of origin to the new environment. In this study, acclimatization was carried out for 14 days to make sure that the mice could adapt to their new environment. The treatment given during acclimatization was the administration of anthelmintics (10 mg/kg

body weight) orally once a day on the 1st and 7th days via oral gavage. Experimental animals were given the antibiotic amoxicillin (15 mg/kg body weight) orally twice a day for five days, from day 2 to day 6, via oral gavage. Animals were also given antiprotozoal sulfamethoxazole (30 mg/kg body weight) orally twice a day from day 8 to day 10. Dosage of drug administration refers to the book of veterinary drugs.15 The treatments given during acclimatization were done to make sure that all mice were free from microorganisms and parasites, such as worms and tick.

The room used for rearing the animals used an air conditioner with temperatures ranging from 22-24°C and humidity of 60-80%. The room was well-lit, quiet, and regularly cleaned once every two days. The experimental animal cages were made of plastic containers with wooden shavings as a base, replaced twice a week. The cages were closed with lids made of woven wire, which was strong, anti-rust, and bite-resistant. The experimental animals were fed using special pellet feed for mice, given twice a day with drinking water sourced from mineral water, and given ad libitum.

This study was done to observe the hepatoprotective effect of date extract on liver cell damage induced by acetaminophen. The animals used were male mice with ddY *strain*. The mice used were 8 weeks old. Experimental animals were divided into 5 treatment groups, each consisting of 5 mice. Mice were divided into dose group 0 as a negative control and groups 1, 2, and 3 as doses administered with commercial date extract in viscous liquid form. The dates used were those on the ripening stage, with a 1460 mg/mL concentration. Group 1 was given 30 µL of dates extract with a dose of 1300 mg/kgBW, and group 2 was given 60 µL of dates extract with a dose of 2900 mg/kgBW; group 3 was given 100 µL of dates extract with a dose of 5000 mg/kgBW, and group 4 as a positive control. All extracts were given using a micropipette for 20 days straight, once a day. The volume of the extract was given based on the treatment group. Groups 0 and 4, as negative and positive control, were not given any preparations. After the 20th day, acetaminophen was induced in the mice with the dosage of 300 mg/kgBW via the intraperitoneal route for two days, in groups 1, 2, and 3 as dose groups and group 4 as positive control group. Group 0, as the negative control group, was not induced by acetaminophen and was used as a normal baseline. Blood was collected on the first 24 hours after induction and on the 26th day after mice were terminated. After termination, the livers of the mice were collected.

A blood examination (hematogram/hematology) was done on day 26. Blood collection was done in every treatment group. The collection of blood samples was done via the intraorbital route. Samples were put inside vacuum tubes containing tripotassium ethylenediaminetetraacetic acid (K3EDTA) anticoagulant. A blood sample was analyzed using a hematology analyzer. A blood chemistry test was also done. Blood biochemistry parameters were serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT). Data were collected in table form using the software Microsoft Office Excel 2019. Data analysis was done using the Statistical Product and Service Solutions (SPPS) with the statistical data normality test using the Kolmogorov-Smirnovv method and ANOVA test,

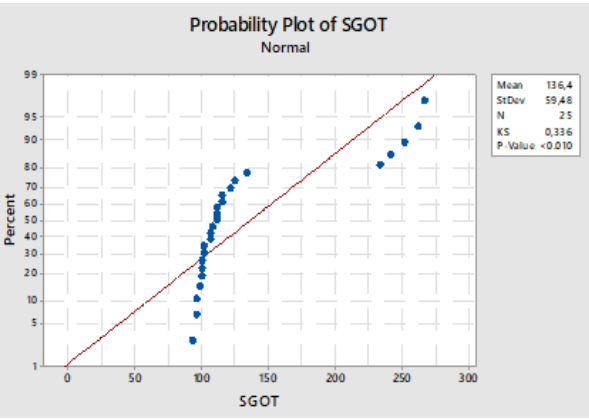

Figure 1 SGOT Data Distribution in A Form of Probability Plot

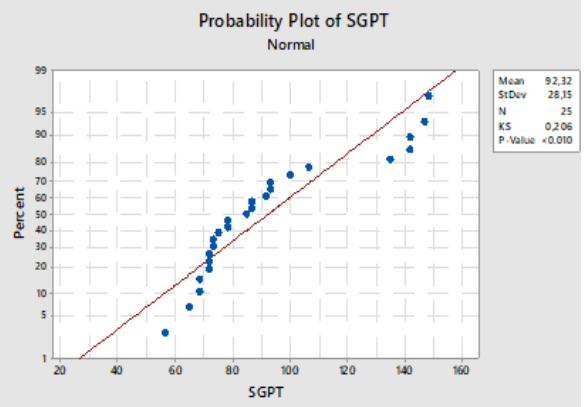

Figure 2 SGPT Data Distribution in A Form of Probability Plot

followed by the Tukey test.

Result

Statistical analysis was carried out to determine the homogeneity and normality of the data. This method was carried out as a reference for the following data analysis stage. This analysis results in the data being normally distributed or not normally distributed. Based on the Kolmogorov test, data that is normally distributed will have a good level of accuracy and interpretation compared to data that is not normally distributed.

The results of the normality analysis of SGPT and SGOT data in this study are presented in Figure 1 and 2. SGPT and SGOT data collected in this study are normally distributed. This refers to the Kolmogorov-Smirnov data's normality coefficient value, which states that the KS value is less than 0.349 for normally distributed data. The KS value for the SGOT data is 0.336, and the KS value for the SGPT data is 0.206. Normally distributed data were suitable for analysis between groups using the ANOVA method and Tukey's follow-up test.

The result done with the ANOVA method showed that mice that were given date extract (K1, K2, K3) had lower serum levels of SGPT and SGOT than mice in the positive group (K4) ($p<0.05$). Figure 3 provides a box plot comparison of each group's average levels of SGOT. The SGOT level in the treatment dose group was shown to be lower than the positive group and was close to the negative group level. The lowest mean SGOT value was found in the negative group, followed by 100 µL treatment dose, 60 µL treatment dose, and 30 µL. The highest value was seen in the positive control. Based on the result, the average level of SGOT decreased along with the increase in the date extract given to the mice. The larger the extract dose, the lower the SGOT level produced.

SGPT levels in mice that were given date extract were lower than in mice in the positive group and closer to the negative group. Graph 4 provides a box plot comparing SGPT levels for each group. The lowest value of SGPT levels was found in the treatment of 100 µL, followed by the negative group, treatment of 30 µL, and treatment of 60 µL. The highest level was seen in the positive group. Based on observations, the average level of SGPT decreased was almost in line with the increase in the dosage of extract

Table 1 The Effect of Date Extract on Levels (µL) of SGPT and SGOT in Mice

Group	SGPT	SGOT
1 (30 µL)	86,0±14,6[a]	114,66 ±13,35[a]
2 (60 µL)	86,3±13,1[a]	111,66±9,35[a]
3 (100 µL)	72,3±12,6[a]	105,32± 7,67[a]
4 (Positive)	142,7±5,2[b]	250,97±13,77[b]
5 (Negative)	74,3 ±4,3[a]	99,39±4,96[a]

*a, b: Different superscript letters on the same line showed significant differences ($p<0.05$)

Figure 3 Effect of the Dosage of Dates Extract on Levels (µL) of SGOT in Mice

given to mice. SGPT level will decrease along with the increase in the dosage.

Table 1 presents data on the levels of SGPT and SGOT in mice that were given the extract, as well as in the positive and negative control groups. The data obtained were tested with a 95% confidence interval and a significance level 0.05 (p=0.05). The test results showed that the levels of SGPT and SGOT were close to the negative group, which was not given any treatment nor induced by acetaminophen. Data analysis of the average value showed a significant difference between the groups that were given the extract and the positive group, which was not given any treatment but was induced by acetaminophen (p<0.05). No significant difference (p>0.05) was seen between the treated mice compared to the negative group. However, a decrease could be seen in the levels of SGPT and SGOT, along with the increase in the dosage.

Discussion

Acetaminophen is a drug with analgesic and antipyretic effects. However, acetaminophen with inappropriate dosage and long-term usage can cause liver damage due to the hepatotoxic property of the drug. Acetaminophen at a toxic dose is biotransformed by cytochrome P450 (CYP2E1 isozyme), producing an unstable, reactive, toxic metabolite, namely N-acetyl-para-benzoquinone imine (NAPQI). Under normal conditions, this metabolite is detoxified by conjugating with glutathione to form mercapturic acid. Administration of acetaminophen in toxic dosage causes many toxic reactive metabolites to form and deplete the glutathione supply

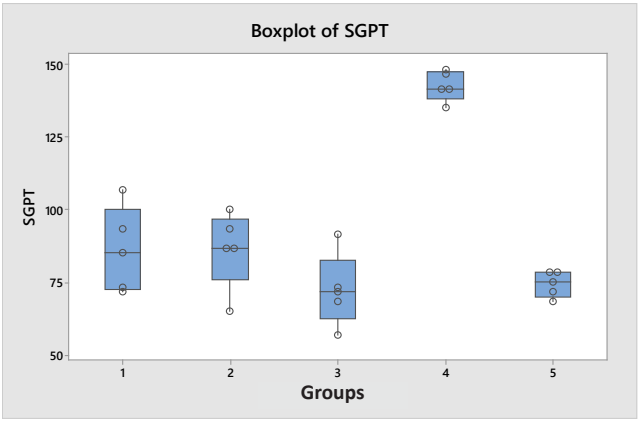

Figure 4 Effect of The Dosage of Dates Extract on Levels (µL) of SGPT in Mice

to conjugate these substances so that the reactive drug metabolites bind to the protein components of liver cells, causing liver damage. Acetaminophen oxidation by cytochrome p450 enzyme also produces free radicals. Suppose free radicals are associated with unsaturated fats such as cell membranes. In that case, there will be lipid peroxidation, which causes damage to the cell membrane structures and functional disorder that will increase serum transaminase levels.[16] Elevation of serum transaminase is one of the strongest signs of cellular leakage and impaired functional integrity of hepatocyte membrane. [17]

Based on the result of the research that has been obtained and statistically analyzed using the ANOVA analysis test, groups that were given the extract show lower SGPT and SGOT levels compared to the positive control or group that was not given any extract ($p<0.05$). The hepatoprotective effect of dates was shown from the level of SGPT and SGOT of the groups that were given the extract. The decrease in the levels of SGOT and SGPT was almost linear with the increase in the extract dosage administered. The decrease in SGOT level followed the increase of the extract dosage, starting from the dosage of 100 µL, followed by the dosage of 60 µL and 30 µL. The SGOT level of mice given the extract showed no significant difference ($p>0.05$) compared to the negative control or mice not given any treatment. On the other hand, the SGOT level of mice that were given the extract showed a significant difference ($p<0.05$) compared to the positive control group. Meanwhile, the decrease in SGPT level was almost in line with the increase in dates extract, with a 100 µL dosage followed by 30 µL and 60 µL. The SGPT level of mice that were given the extract did not show any difference ($p<0.05$) compared to the negative control group.

The results showed that the date extract had a hepatoprotective effect as seen from the comparison between SGPT and SGOT levels in the treatment group with the negative control group, followed by the positive control. This is due to the components contained in the dates. Dates can be consumed as fresh fruit or called the besser stage (short shelf life), or tamer stage (long shelf life). Analysis of phytochemical composition during different ripening stages showed that the highest number of polyphenolic compounds, carotenoids, and anthocyanins were found in the early stages (kimri and besser) and decreased during directed ripening stages showed that the highest number of polyphenolic compounds, carotenoids, and anthocyanins were found in the early stages (kimri and besser) and decreased during direct ripening. The besser stage was found to be rich in flavonoids, tannins, and phenolic acids such as ferulic acid, caffeine, p-coumaric, and protocatechuic acids and catechins, which are rich in antioxidant activity.[18] Antioxidant content is believed to reduce free radicals and reduce oxidative stress levels. This has an impact on the repair of a damaged liver. Other polyphenols found in dates are catechins, quercetin, and luteolin. Based on research conducted by Abdelaziz and Ali,[19] the administration of this polyphenol content in distilled water has been shown to improve liver lesions in the form of vacuolization and fibroblast proliferation in the liver of rats.

Other than having an antioxidant effect, dates also have anti-inflammatory activity through the downregulation of cyclooxygenase-1 and 2 (COX-1 and COX-2) enzymes. This activity is attributed to compounds rich in polyphenols, namely glycosides, and flavonoids.[20] Apart from inhibiting the COX-1 and COX-2 enzymes, dates also can inhibit the CYP2E1 enzyme. The CYP2E1 enzyme is an enzyme that can be found in hepatocytes and functions to metabolize molecules such as ethanol, acetaminophen, and pro-carcinogens. CYP2E1-mediated metabolism produces toxic intermediates and excessive amounts of ROS. High ROS levels due to this enzyme's activity are the main cause of various liver diseases, especially those caused by chronic alcohol consumption.[21]

Based on the GC-MS analysis conducted by Nehdi et al.,[22] dates contain several saturated and unsaturated fats, such as stearic acid, palmitic acid, 9-octadecenoic acid, fumaric acid, and -linolenic acid. 9-octadecenoic acid, another name for oleic acid, is classified as an anti-inflammatory agent because of its inhibitory effect on pro-inflammatory signals. The presence of linoleic acid in dates can reduce oxidative stress caused by injury to liver lesions, liver steatosis, and non-alcoholic liver disease.[23]

Based on the results of the study, the administration of date extract was proven to have a hepatoprotective effect on mice. This is evidenced by the significant decrease in SGPT and SGOT levels compared to the positive control group and the levels of SGPT and SGOT, which were not so different from the negative control group. Further research must be done to utilize dates and their contents as hepatoprotective agents.

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RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n3.3287
Majalah Kedokteran Bandung. 2023;55(3):161–170

Majalah Kedokteran Bandung (MKB)

Received: March 14, 2023
Accepted: June 16, 2023
Available online: September 30, 2023

In-silico study of the Effectiveness of *Allium sativum L.* extract as an Angiotensin-Converting Enzyme (ACE) Inhibitor in Hypertension

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Abstract

Over the last decade, the global prevalence of hypertension rate has increased by 5.2% and, in Indonesia, the prevalence rate has increased significantly from 25.8% in 2013 to 34.1% in 2018. Hypertension treatments using blood pressure-lowering drugs, such as angiotensin-converting enzyme (ACE) inhibitors, often cause unpleasant side effects. These side effects increase the interest in using potentially effective natural remedies, such as garlic. This study aimed to determine which organosulfur compounds in garlic can act as an ACE inhibitor to reduce blood pressure in hypertension using a cheminformatics approach. Eighteen organosulfur compounds of Allium sativum L. were screened based on Lipinski's rules and ADMET evaluation. Seven compounds passed the screening and were subjected to QSAR analysis, molecular docking analysis, and molecular dynamics simulations to assess the stability of the protein. The seven compounds then underwent molecular docking and QSAR analysis. Ajoene (4,5,9-trithiadodeca-1,6,11-triene-9-oxide) and S-allylmercaptocysteine (SAMC) were two compounds with better docking values compared to the positive control compound. The QSAR analysis also showed that SAMC had an activity as an ACE inhibitor. The ADMET evaluation showed that Ajoene and SAMC had good absorption and could not penetrate the blood-brain barrier. Molecular dynamics simulation of ACE complexes Ajoene, SAMC, and Captopril ranged from 0.05 to 5.61 Å but exhibited a pattern of synonymous fluctuations for most residues. Based on the simulation data, the organosulfur compounds from garlic, Ajoene, and SAMC are proven to have a mechanism of action as ACE inhibitors to reduce blood pressure in hypertension.

Keywords: ACE inhibitors, cheminformatics, garlic, hypertension

Introduction

Hypertension is a significant risk factor for cardiovascular disease (CVD), a leading cause of morbidity and mortality worldwide.[1] Hypertension management and control are critical to preventing cardiovascular disease and other diseases. Over the last decade, the global prevalence rate has increased by 5.2%, and in Indonesia, the prevalence of hypertension has increased significantly from 25.8% in 2013 to 34.1% in 2018. [2,3]

Hypertension or high blood pressure is defined as having arterial blood pressure above the normal value. According to the Indonesian Ministry of Health, normal blood pressure for systolic blood pressure is less than 120 mmHg and less than 80 mm Hg for diastolic blood pressure.[1] Based on these guidelines, a person is said to have hypertension if the systolic blood pressure is above 140 mmHg and/or the diastolic blood pressure is above 90 mmHg.[3]

Hypertension treatments using blood pressure lowering drugs often cause various unpleasant adverse effects.[4] The most common adverse effect is dry cough due to the accumulation of bradykinin in respiratory tissues due to angiotensin-converting enzyme (ACE) inhibition.[4] It also helps to explain the growing interest in potentially effective natural remedies, such as garlic. Garlic (*Allium sativum L.*) has been used as a spice, food and remedy for centuries. There are several ways to consume garlic for treatment, such as raw garlic, freshly cooked garlic, garlic oil, garlic powder, and aged garlic extract. Research conducted by Reid et al. showed that garlic supplementation to hypertensive patients can reduce blood

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pressure by 10 mmHg.[4] The result might be due to the organosulfur compounds found in garlic can intervene in the pathway that causes hypertension.[4,5]

Although several mechanisms cause hypertension, this research will only focus on the mechanism of organosulfur compounds from garlic as angiotensin-converting enzyme (ACE) inhibitors, which regulate blood pressure through the renin-angiotensin-aldosterone system (RAAS) in the body.[7] The RAAS system, composed of three major components: renin, angiotensin II, and aldosterone, is a critical regulator of blood pressure in our body. Once renin has been released into the blood, it cleaves its target, angiotensinogen, into angiotensin I. Angiotensin I is initially inactive but acts as a precursor to angiotensin II. The conversion of angiotensin I to angiotensin II is catalyzed by an enzyme called angiotensin-converting enzyme (ACE). After angiotensin I is converted into angiotensin II, it affects the kidney, adrenal cortex, arterioles, and brain by binding to angiotensin II type I (AT1) and type II (AT2) receptors to coordinate in regulating blood pressure in the body.[7]

This study uses a cheminformatics approach to determine which organosulfur compounds in garlic can act as an ACE inhibitor to reduce blood pressure in hypertension.

Methods

The ACE sequences were obtained by searching the NCBI database using RefSeq and *Homo sapiens* filters. Protein modeling of the sequence data will be in FASTA format using the SWISS-MODEL server (https://swissmodel.expasy.org/), and the parameter used for modeling will be QMEANDisCo.[8,9]

The organosulfur compounds of *Allium sativum L.* had been collected from several databases such as IJAH (Indonesia Jamu Herbs) and Herbal DB UI, which were expected to have potential as ACE inhibitors.[10,11] The SMILES structures of these organosulfur compounds were obtained from PubChem, and the SWISSADME (http://www.swissadme.ch/index.php) web server was used to evaluate each ligand's molecular properties and drug-likeness.[12] All drug-likeness parameters in SWISS-ADME were used to filter out organosulfur compounds found in garlic.[13] The ligands rejected by more than two drug-likeness parameters would be excluded from the molecular docking analysis.

The toxicity of the ligands was also analyzed using ADMET lab 2.0 (https://admetmesh.scbdd.com/) web server. Several parameters were analyzed, such as BOILED-EGG, AMES toxicity, skin sensitization, carcinogenicity, and eye irritation.[14,15] The ligands that have passed the toxicity parameter will be utilized for molecular docking prediction.

The QSAR analysis was conducted to screen the bioactivity of the chemical molecules. The analysis was conducted using the Prediction of Activity Spectra for Substances (PASS) tool provided by the Way 2 drug / PASS server (http://www.way2 drug.com/PASSOnline/). The PASS tool provided by this server would be able to predict whether the organosulfur compounds derived from Garlic would have inhibitory activity against the ACE. The output from this server would be a tabulation of data related to the interaction of chemical compounds with biological entities.[16]

The surface area and binding site of the angiotensin-converting enzyme (ACE) would be identified by using the Computed Atlas of Surface Topography of Proteins (CASTp) server (http://sts.bioe.uic.edu/castp). Delaunay triangulation, alpha shape, and discrete flow were the parameters used to measure the surface area and binding site of ACE protein.[17]

Molecular docking of the selected ligands and ACE was performed using the PatchDock (http://bioinfo3d.cs.tau.ac.il/PatchDock/) server. There are two parameters evaluated in the molecular docking simulation: docking score and atomic contact energy score (ACE score).[18] The docking score measures the strength of ligand–protein interactions, with higher scores suggesting more favorable interactions and higher affinity.[18] The ACE score evaluates the energy contribution associated with the ligand–protein atomic interactions, with a lower ACE score suggesting a more stable and energetically favorable ligand-protein complex.[18] To validate the molecular docking method, the docking results of organosulfur compounds with ACE had to have a higher docking score and ACE score than the reference compound. In this study, Captopril was used as the reference compound of the ACE inhibitor compared to the organosulfur compounds present in garlic.[4] The results of the molecular docking analysis will be used as input data in the molecular dynamic simulation.

MD simulation of the selected ligands and ACE was performed using the CABSFLEX2 server (http://biocomp.chem.uw.edu.pl/CABSflex2/submit) server.[19] This server runs MD

simulations with parameters such as length of the simulation, time step, temperature, number of cycles, and cycles between trajectory frames with default settings to predict the flexibility of the ACE ligand and molecule.[19] The positions of atoms in proteins are tracked over time, enabling the calculation of root mean square fluctuation (RMSF), which measures the degree of deviation from an average position. The RMSF analysis provides information on the ligand's and enzyme's flexibility and is critical for investigating the interactions between organosulfur compounds and ACE molecules.[18] The expected RMSF threshold value between ligands and proteins in MD simulation would be around 1–3 Å.[18] The conformation between organosulfur compounds and ACE is considered stable in this value range.

Results

The ACE sequence data was obtained from the NCBI database with access codes NP_001171528.1 in FASTA format, and then the data was inputted into the SWISS-MODEL server for modeling simulation. The output of SWISS-MODEL was an ACE protein model with a template of PDB ID 4C2O, with a GMQE value of 0.99, a Global QMEANDisCo of 0.93±0.05, and a sequence identity of 99.83%. The homology modeling between the ACE model and the co-crystal structure 1UZF is shown in Figure 1.

To validate the model given by SWISS-MODEL, we compared it to PDB ID 1UZF, an ACE with Captopril in its structure, as shown in Figure 1. The homology modeling results show the RMSD value of the model, and 1UZF is 0.35 Å. The similarity between them is 99.5%, suggesting that the ACE model given by SWISS-MODEL closely resembles the 1UZF model.

From the three herbal medicine databases, 56 chemical substances were retrieved. This study combined the data and removed the non-organosulfur compounds. The three databases yielded eighteen organosulfur compounds, as

Table 1 The Eighteen Organosulfur Compounds are Mostly in Garlic (*Allium Sativum* L.).

Compound Name	SMILES Structure	PUBCHEM ID
Diallyl sulfide	C=CCSCC=C	11617
Diallyl disulfide	C=CCSSCC=C	16590
Diallyl trisulfide	C=CCSSSCC=C	16315
Alliin	C=CCS(=O)CC(C(=O)O)N	87310
Ajoene (4,5,9-trithiadodeca-1,6,11-triene-9-oxide)	C=CCSSC=CCS(=O)CC=C	5386591
Allicin	C=CCSS(=O)CC=C	65036
Methyl propyl disulfide	CCCSSC	16592
Methylselenocysteine	C[Se]CC(C(=O)O)N	147004
Allyl methyl disulfide	CSSCC=C	62434
S-Methyl-L-cysteine sulfoxide	CS(=O)CC(C(=O)O)N	182092
Allyl methyl sulfide	CSCC=C	66282
S-Methylthiocysteine	CSSCC(C(=O)O)N	3080775
S-allylmercaptocysteine (SAMC)	C=CCSSCC(C(=O)O)N	9794159
y-L-Glutamyl-S-allyl-L-cysteine	C=CCSCC(C(=O)O)NC(=O)CCC(C(=O)O)N	11346811
y-L-Glutamyl-S-1-propenyl-L-cysteine	CC=CSCC(C(=O)O)NC(=O)CCC(C(=O)O)N	87289205
Methyl propyl disulfide	CCCSSC	16592
S-1-Propenyl-L-cysteine	CC=CS(=O)CC(C(=O)[O-])[NH3+]	90657185
2-vinyl-4H-1,3-dithiin	C=CC1SCC=CS1	133337

Figure 1 The Homology Modelling Between ACE model generated from SWISS-MODEL (gray) and PDB ID: 1UZF (violet). The captopril ligand originated from 1UZF (green)

listed in Table 1.

The eighteen compounds were then analyzed for their physicochemical properties and drug-likeness using the SWISS-ADME server, and the compounds that received a rejection of more than two drug-likeness parameters, as shown in Table 2, would be eliminated for molecular docking analysis. As a result, two compounds, γ-L-Glutamyl-S-allyl-L-cysteine and γ-L-Glutamyl-S-1-propenyl -L-cysteine, are eliminated.

The compounds that had passed the selection would be analyzed for toxicity. The combined ADMET and Boiled-Egg analysis showed that seven compounds in Table 3 were safe to use as lead compounds for ACE inhibitors.

Furthermore, we conducted a QSAR study to investigate whether any of the seven compounds had the potential to become ACE inhibitors. The results of the QSAR analysis showed that three organosulfur compounds from garlic had the potential as ACE inhibitors, as shown in Table 4. Among the three compounds, SAMC has the highest bioactivity as ACE inhibitors, indicating that SAMC can potentially be an inhibitor of the ACE protein.

Before the molecular docking simulation between seven compounds and ACE was carried out, the position of the binding pocket of the protein was analyzed. The calculation result

Table 2 SWISS-ADME Drug-Likeness Analysis of Organosulfur Compounds in Garlic

Compound Name	Druglikeness				
	Lipinski	Ghose	Veber	Egan	Muege
Diallyl sulfide	Yes	No	Yes	Yes	Yes
Diallyl disulfide	Yes	No	Yes	Yes	No
Diallyl trisulfide	Yes	No	Yes	Yes	No
Alliin	Yes	Yes	Yes	Yes	No
Ajoene	Yes	Yes	Yes	Yes	Yes
Allicin	Yes	No	Yes	Yes	No
Methyl propyl disulfide	Yes	No	Yes	Yes	No
Methyl selenocysteine	Yes	No	Yes	Yes	No
Allyl methyl disulfide	Yes	No	Yes	Yes	No
S-Methyl-L-cysteine sulfoxide	Yes	No	Yes	Yes	No
Allyl methyl sulfide	Yes	No	Yes	Yes	No
S-Methylthiocysteine	Yes	No	Yes	Yes	No
S-allylmercaptocysteine (SAMC)	Yes	Yes	Yes	Yes	No
γ-L-Glutamyl-S-allyl-L-cysteine	Yes	Yes	No	No	No
γ-L-Glutamyl-S-1-propenyl-L-cysteine	Yes	Yes	No	No	No
Methyl propyl disulfide	Yes	No	Yes	Yes	No
S-1-Propenyl-L-cysteine	Yes	No	Yes	Yes	No
2-vinyl-4H-1,3-dithiin	Yes	No	Yes	Yes	No

Table 3 ADMET Analysis of Organosulfur Compounds That are Present in Garlic. The output Value of ADMET Analysis is in the Range of 0 to 1 and is Converted into the Following Categories 0-0.1 (---); 0.1-0.3 (--); 0.3 - 0.5 (-);0.5-0.7 (+); 0.7 - 0.9(++); 0.9-1.0(+++).

Compound Name	ADMET Analysis						
	BBB permeable	HIA	LC_{50}	AMES Toxicity	Skin Sensitization	Carcinogenicity	Eye irritation
Alliin	—	+++	3.515	+	–	–	–
Ajoene	—	+++	5.774	+++	+++	++	+++
Methyl selenocysteine	—	+++	3.259	++	-	–	–
S-Methyl-L-cysteine sulfoxide	—	+++	2.756	–	–	++	—
S-Methylthiocysteine	—	+++	3.785	–	+	–	–
S-allylmercaptocysteine (SAMC)	—	+++	4.259	–	++	–	–
S-1-Propenyl-L-cysteine	—	+++	3.972	+	-	+	—

from the CASTp server showed the surface area and volume of the binding pocket of the ACE protein, as shown in Figure 2.

In molecular docking analysis, Captopril was used as the reference compound of the ACE inhibitor since it had been approved as a drug to treat hypertension. The PatchDock output results in Table 5 showed that two garlic-derived compounds, Ajoene and SAMC, have a higher docking value than the reference compound.

In addition to the docking analysis, the researchers also analyzed and visualized the interactions between the ligands and proteins of the seven organosulfur compounds against the captopril molecule. The results are presented in Table 6 and Figure 3.

The molecular dynamics simulation of Ajoene with ACE protein showed a stable conformation because most of the ACE protein residues had RMSF values between the RMSF thresholds of 1–3 Å. Similarly, SAMC showed a stable conformation because it only had a slight residue of ACE protein with an RMSF value falling above the threshold value. The results of the RMSF graph of the two compounds can be seen in Figure 4.

Discussion

According to the 2022 global burden report, hypertension is the leading cause of cardiovascular disease and premature death

Table 4 The PASS Results from Organosulfur Compounds in Garlic

Compound	ACE inhibitor bioactivity
Alliin	No
Ajoene	No
Methyl selenocysteine	No
S-Methyl-L-cysteine sulfoxide	Yes; Pa = 0.060, Pi = 0.038
S-Methylthiocysteine	Yes, Pa = 0.095, Pi = 0.017
S-allylmercaptocysteine (SAMC)	Yes, Pa = 0.104, Pi = 0.015
S-1-Propenyl-L-cysteine	No

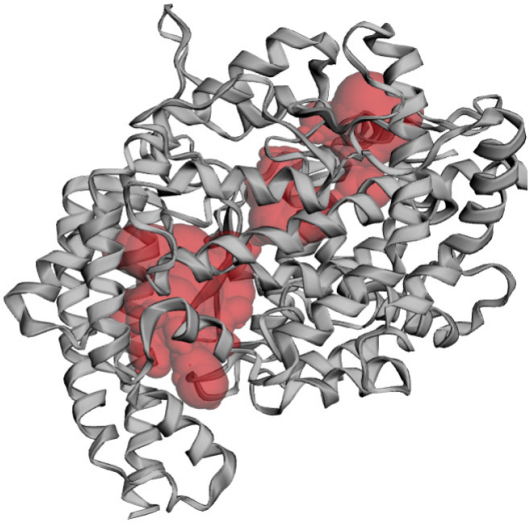


Figure 2 The ACE Model Generated from SWISS-MODEL server. The red Color Shows the Binding Pocket of ACE

worldwide. It is estimated that the number of hypertension cases has increased significantly in low and middle-income countries (LMICs).[1] However, global mean blood pressure (BP) has remained constant or decreased slightly over the past four decades due to the widespread use of antihypertensive drugs. Although antihypertensive medications can lower blood pressure, they frequently have unpleasant unpleasant adverse effects.[4] As a result, people are looking for alternative treatments for hypertension, using natural remedies like garlic (*Allium sativum L.*).

In this study, we will try to explain the mechanism of action of the active compounds in garlic in lowering blood pressure, one of which is by inhibiting the action of ACE in regulating blood pressure in the body. A virtual screening will be conducted to determine whether organosulfur lead compounds from garlic can reduce blood pressure in hypertensive patients. Therefore, this study aims to determine whether garlic's organosulfur compounds can reduce blood pressure in hypertensive patients by inhibiting ACE proteins, similar to captopril compounds.

L-cysteine sulfoxides and γ-glutamyl-L-cysteine peptides are garlic's two main organosulfur compounds.[6] Alliinase is released when garlic is crushed or chopped, which catalyzes the formation of Allicin from S-allyl-L-cysteine sulfoxide (Alliin), and Allicin degrades quickly into a variety of organosulfur compounds.[6]

The QSAR analysis was performed to screen the bioactivity of the organosulfur compounds from garlic by using the Prediction of Activity Spectra for Substances (PASS) tool. Its algorithm predicts the probability of activity (Pa) and the probability of inactivity (Pi) of the organosulfur compounds from garlic as ACE inhibitors based on their known structure-activity relationships database.[16] The Pa and Pi values provide an indication of the likelihood of a compound exhibiting or lacking a specific activity, respectively, as predicted by the PASS algorithm.[16] By default, the Pa=Pi value is set as the PASS threshold, so all compounds with Pa>Pi are suggested to have biological activity.[16] The average prediction accuracy is estimated to be about 95% accurate.[16] The QSAR analysis results

Table 5 The Molecular Docking Prediction of Selected Ligands and ACE Using The PatchDock Web Server

Ligand	Docking Score	Atomic Contact Energy score (ACE score)
Captopril	3642	-34.72
Ajoene*	4452	-159.33
Alliin	3132	-48.53
Methyl Selenocysteine	2756	-66.99
S-1-Propenyl-L-cysteine	3134	-48.53
S-allylmercaptocysteine (SAMC)*	3704	-99.93
S-Methyl-L-cysteine sulfoxide	2750	-146.86
S-Methylthiocysteine	2944	-78.44

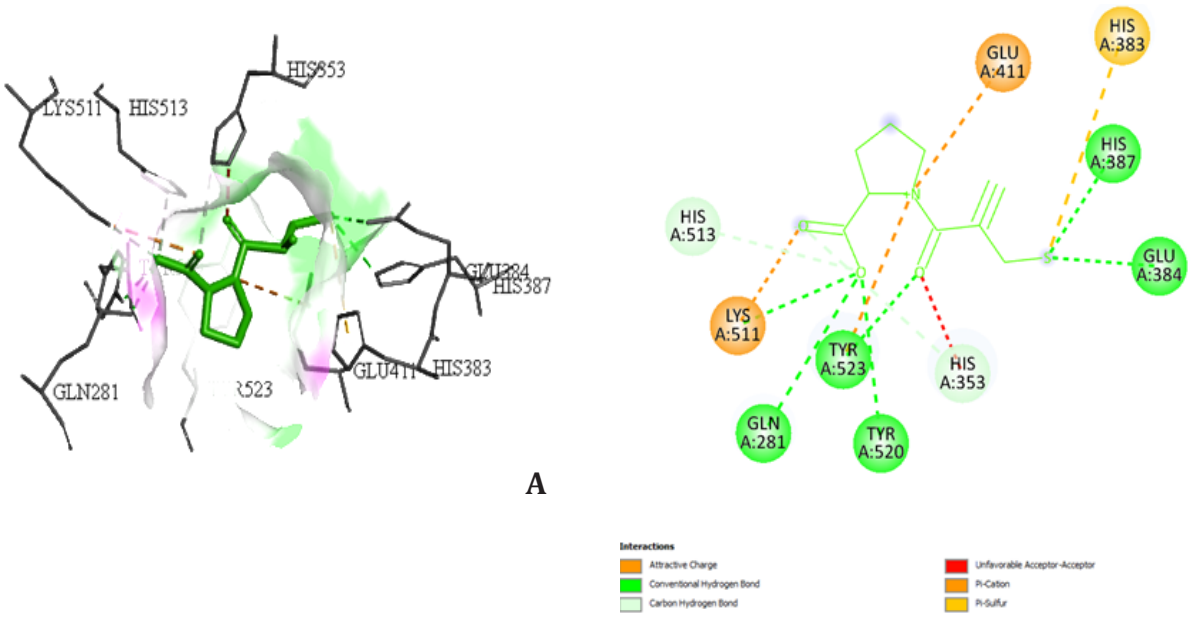
* The most favorable docking score and ACE score

Table 6 The Organosulfur Compound Interaction with ACE

Compound Name	Organosulfur Compound Interaction with ACE			
	Hydrogen Bonds	Salt Bridges	Hydrophobic Interactions	Total interactions
Captopril	GLN281	HIS353, LYS511, HIS513	PHE457	5
Ajoene*	HIS353, HIS513	GLU384, GLU411	VAL379	5
Alliin	HIS353, GLU384, HIS387, GLU411, HIS513	GLU411	ALA354, VAL380	8
Methyl Selenocysteine	HIS353, ALA354, GLU384	HIS383, HIS387		5
S-1-Propenyl-L-cysteine	ALA356, HIS387, GLU411	HIS387	TYR520, TYR523	6
SAMC*	ALA356, GLU384, HIS387, GLU411	HIS383, HIS387	PHE457, TYR523, PHE527	9
S-Methyl-L-cysteine sulfoxide	HIS353, ALA354, ALA356, HIS513	GLU384, HIS387, GLU411		7
S-Methylthiocysteine	HIS353, GLU411, HIS513, TYR523	HIS383, HIS387		6

in Table 4 show that three compounds have the potential to be ACE inhibitors.

Furthermore, we used molecular docking to investigate how these organosulfur compounds form complexes with ACE proteins. Molecular docking was used to perform virtual screening of the active compounds from garlic, to rank the results, and to propose structural hypotheses about how these ligands inhibit ACE proteins, which is very useful in lead optimization of these organosulfur compounds. Although Ajoene had no activity against the ACE protein in QSAR analysis compared to SAMC, the docking simulation results showed that Ajoene had a



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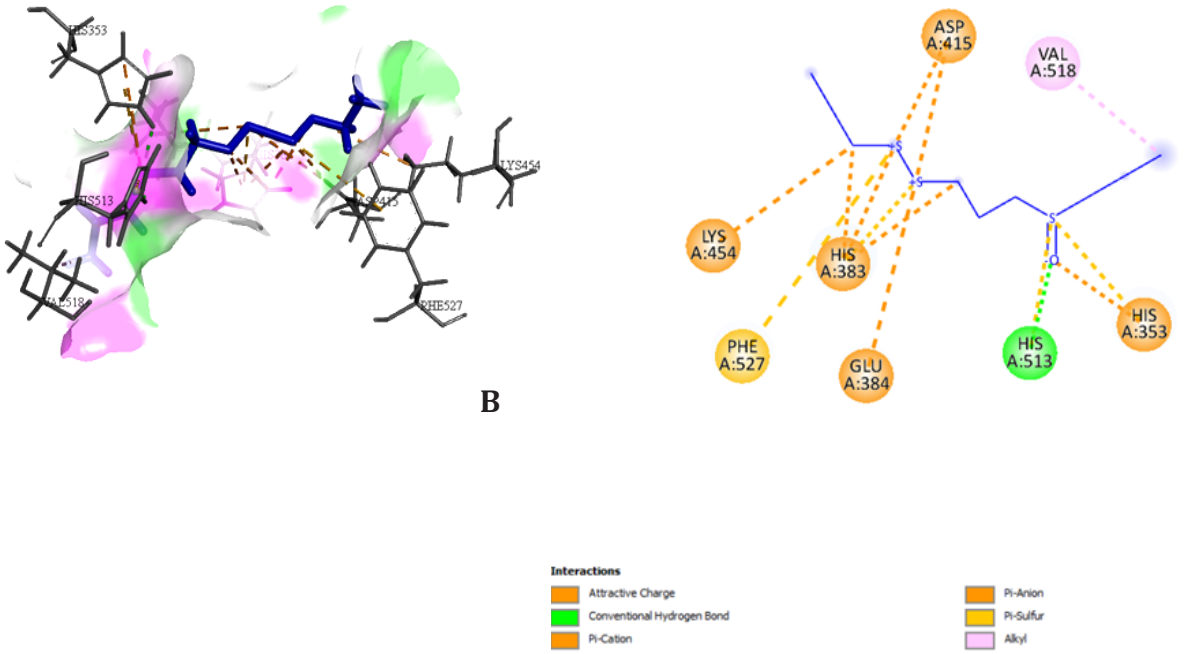

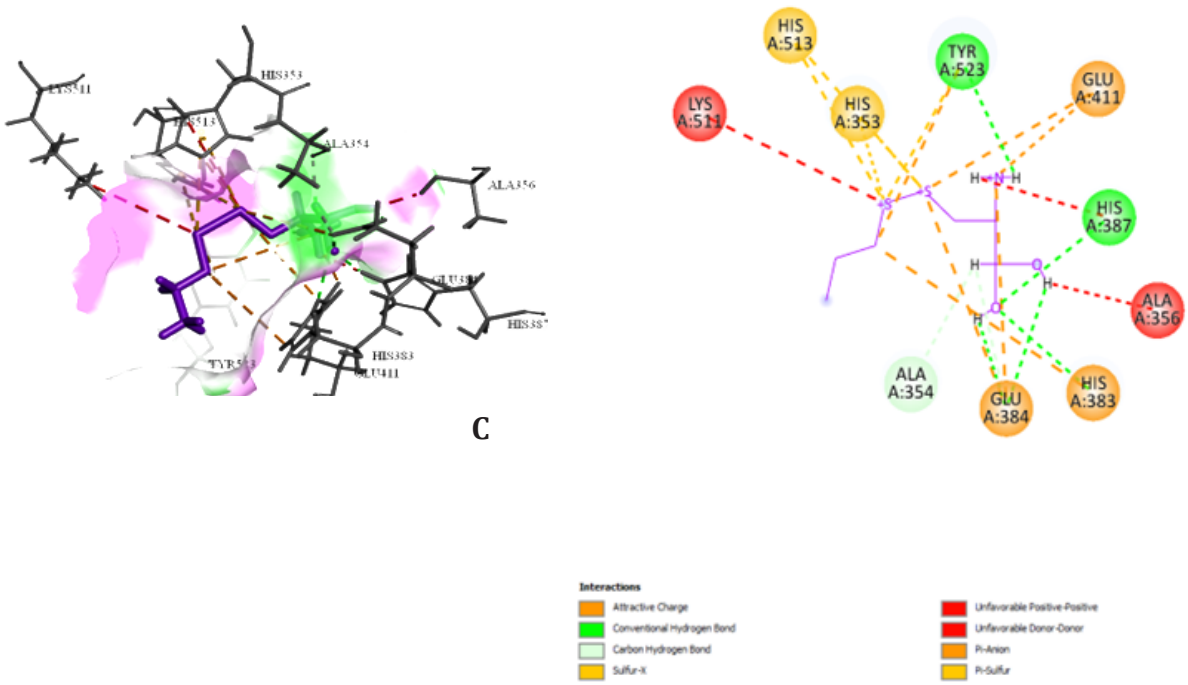

Figure 3 Molecular interaction between ACE and (A)captopril (yellow); (B) Ajoene (blue); and (C)SAMC (violet) in its binding site. Figures were visualize using Discovery Studio 2021

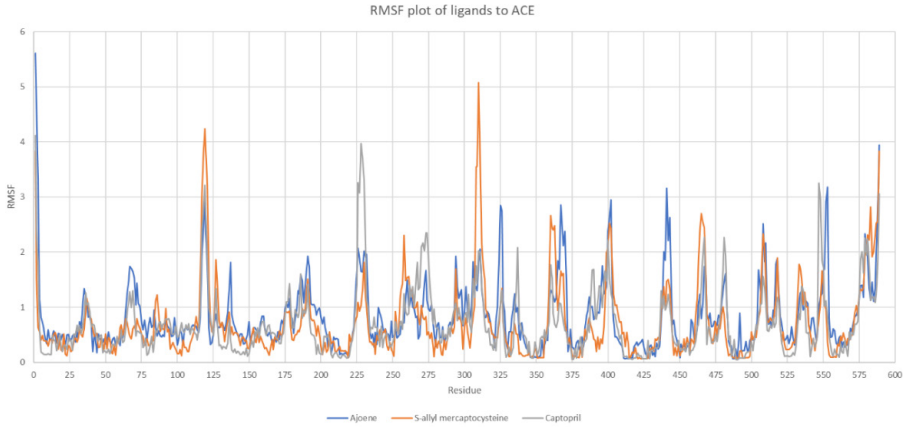

Figure 4 RMSF plot of Ajoene (blue), SAMC (orange) and Captopril (grey) with an Angiotensin-Converting Enzyme (ACE)

higher docking score than Captopril and SAMC.

Based on the docking simulation results, MD simulations were carried out to analyze the dynamic interactions between ACE and ajoene, SAMC, and Captopril. Figure 2 depicts a superimposed graphical representation of the residue-based Root Mean Square Fluctuations (RMSF) analysis of 625 residues in the ACE protein complexes to Ajoene, SAMC, and Captopril. The RMSF value was used to calculate the magnitude of each residue's fluctuations. The RMSF of the three complexes varies greatly, ranging from 0.05 to 5.61 Å. Although the interaction between ACE and ajoene, SAMC, and Captopril has a wide range of fluctuations, the superimposition of the RMSF values of the Ajoene, SAMC, and Captopril complexes onto the ACE protein also exhibits a pattern of synonymous fluctuations for the majority of the residues. This suggests that Ajoene and SAMC can bind to the ACE protein's binding pocket via a mechanism similar to Captopril.

The outcomes of this molecular docking simulation are consistent with the outcomes of the randomized controlled trials. Garlic preparations reduced systolic blood pressure (SBP) by 8.7 mm Hg and diastolic blood pressure (DBP) by 6.1 mm Hg compared to medications at standard dose, which reduced SBP by 9.1 mm Hg and DBP by 5.5 mm Hg.[4] Ajoene is one of garlic's most important natural compounds derived from Allicin. Ajoene is rapidly metabolized and excreted from the body, even after large amounts of garlic (up to 25 grams) or 60 mg of pure Allicin are consumed (in tablet preparations).[20] Meanwhile, SAMC is metabolized and excreted from the body for a longer time than Ajoene. These observations showed that these compounds might play a key role in the biological activity of garlic.[20]

The limitations of this study arose from the fact that it was not conducted directly on individual patients but instead relied on existing data in the database and the results of other people's meta-analyses. However, the results of our in-silico analysis align with the data from other researchers' meta-analyses, which strengthens our hypothesis that there are organosulfur compounds in garlic, namely Ajoene and SAMC, have the exact mechanism of action as antihypertensive drugs, and this can be a suggestion for more in-depth molecular research.

In concusion, that garlic consumption effectively lowers blood pressure with minimal side effects in people with hypertension. Organosulfur compounds from garlic, Ajoene, and SAMC have a mechanism of action as ACE inhibitors to regulate blood pressure, which is similar to antihypertensive drugs.

Furthermore, the findings in this study could be a valuable source of information for the development of natural-based antihypertensive drugs.

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RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n3.3328
Majalah Kedokteran Bandung. 2023;55(3):131–141

Majalah Kedokteran Bandung (MKB)

Received: April 29 2023
Accepted: September 22, 2023
Available online: September 30, 2023

Knowledge and Attitude of Short Stature and Its Treatment in Saudi Arabia

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Abstract

Both tall and short parents are concerned about their children's short stature. This study aimed to explore the knowledge about short stature (SS) and attitude towards its treatment among the general public of Saudi Arabia. This was a cross-sectional study that utilized an online-administered questionnaire distributed between August 2021 and March 2022. Binary logistic regression was conducted to identify factors that affect participants' knowledge. This study included a total of 6,852 individuals. The vast majority of the participants (77.5%) expressed satisfaction with their height. A total of 40.4% of participants stated that they were aware of a treatment option for their low height. With a mean score of 13.0 (SD:5.8) out of 25, the participants demonstrated a moderate level of knowledge of short stature (52.0%). The majority of participants (78.0%) stated that if they have a problem with short stature or want to enhance their own or their children's height, they are willing to consult a doctor about it. Participants living in the northern and eastern areas, those with bachelor degree, and those working in the healthcare field were more likely to be knowledgeable about short stature compared to others ($p \leq 0.01$). Saudi Arabians have a moderate understanding of SS, which needs to be improved. Campaigns to increase the general public's and parents' knowledge about SS, which is ultimately connected to earlier diagnosis and better management outcomes, are needed. Additional research is required to examine the most effective strategies for raising public knowledge of SS.

Keywords: Attitude; knowledge, Saudi Arabia, short stature, survey

Introduction

Growth is an ongoing biological process influenced by genetic, nutritional, environmental, and hormonal factors.[1] Short stature (SS) is described as being shorter than the third percentile of the population.[2] The diagnosis of short stature necessitates biochemical and radiological tests, such as nutritional evaluation, hormonal evaluation, and bone age estimation.[1] Growth is influenced by Ethnicity, lifestyle, diet, culture, and socioeconomic variables. As a result, the causes of SS in children in underdeveloped nations differ from those in rich countries.[2] Short stature can be detected using two methods. Firstly, SS is defined as a height less than 2 standard deviations (SD) for age and gender in the population or less than 2 SD of mid-parental height (MPH). Secondly, through serial growth monitoring, Growth faltering is defined as crossing two centiles on a growth curve in the wrong direction.[2] In a previous study in India, 2.86% of children had SS.[3] In another study that was conducted in Egypt, the prevalence of SS was estimated to be around 17.0% and their main aetiologies were familial (40.8%) and constitutional (24.2%).[4]

One in every 3,500 people had growth hormone deficiency (GHD) (defined as a peak GH response to stimulation of less than 10 ng/dL).[4] Familial Short Stature (FSS), Constitutional Growth Delay (CGD), and GHD have been identified as the most common causes of SS in investigations worldwide. The modest discrepancy in SS prevalence reported by different studies is attributable to the different levels and types of health-care institutions [2]. Both tall and short parents are concerned about their children's short stature. Academics, career placement, leadership and performance, sports participation, and entrance into the glamour world have all been connected to the importance of height or stature. Depending on the height deficiency and the child's coping capacities, psychosocial stress connected with shortness is more stressful.[5]

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With the development in the Saudi population's economic situation and style of living, more parents are concerned about their children's growth. According to a previous survey in Saudi Arabia, SS is one of the most prevalent referrals to an adolescent endocrine clinic in Saudi Arabia.[6] A previous study in Saudi Arabia by El Mouzan et al.[6] reported that in comparison to the global prevalence of SS, the prevalence of short stature in Saudi children and adolescents is intermediate. To the best of our knowledge, previous studies in Saudi Arabia have yet to look into the Saudi population's knowledge of short stature and attitudes toward treatment. Exploring the general public's knowledge of short stature is critical because it aids in early diagnosis of the condition and seeking medical assistance, which is ultimately linked to better health outcomes for managed patients. This information can help with more accurate diagnosis, better handling related health problems, and creating focused solutions. This study aimed to explore the knowledge about SS and attitudes towards its treatment among the general public in Saudi Arabia.

Methods

Between August 2021 and March 2022, a cross-sectional study was conducted utilizing an online-administered questionnaire. The questionnaire link was distributed through social media platforms (Facebook, Twitter, Snapchat, and Instagram) in order to reach a wider variety of participants from various sociodemographic groups. The study's aims and inclusion criteria were clearly mentioned in the questionnaire's cover letter. Before beginning the questionnaire, the participants were asked to provide their consent to take part. Participants who consented to offer their consent were told to begin filling out the questionnaire; otherwise, the survey was closed.

A convenient sampling technique was employed to invite the participants who met the inclusion criteria. All Saudi Arabians above the age of 18 and living in Saudi Arabia who were willing to participate were included in the study. Individuals under 18, who lived outside of Saudi Arabia, had mental problems, did not understand the Arabic language, and did not consent to participate were, however, excluded.

The study instrument was divided into three main sections and 33 questions (multiple choice and yes/no format), and it was based on a thorough evaluation of the literature. The first section, which consists of ten items, looks into the sociodemographic characteristics of the study participants and whether they have heard about bone-lengthening operations. The second section consisted of five questions that probed their perceptions of their height compared to others and their concept of the ideal height. The third portion examined participants' understanding of short stature (13 items) and attitudes regarding treatment (5 items). The knowledge items covered the causes of short stature, clinical symptoms, diagnostic procedures, and treatment strategies. A consultant in orthopedics reviewed the questionnaire instrument for comprehensibility and understandability, and he confirmed it. The questionnaire was translated into Arabic and tested on a random sample of our study population to identify any concerns with context and content.

The questionnaire tool was evaluated and approved by consultant medical doctors. Feedback from experts and other knowledgeable individuals was gathered to determine the content validity. They were requesting their evaluation of the questionnaire and expert opinion on whether or not the questions adequately capture the intended construct or topic. Face validity was examined by the researchers and other experts evaluating the items. This process consisted of determining whether the queries appeared to measure what they were intended to measure. This process involved ensuring that the items are explicit, relevant, and suitable for the being assessed construct. They stated the questionnaire was easy to comprehend and complete. Furthermore, before the questionnaire was used on a wider scale, pilot research with a small group of participants was carried out to evaluate comprehension, and they confirmed that it was simple and clear.

The research ethics committee of the author's affiliated institution (HAPO-01-R-011, Ref: 197/2022) reviewed and approved the study, which follows the rules of the National Committee of Bio-Ethics. The data analyses were carried out using the statistical package for the social sciences, version 27 (SPSS, Armonk, NY: IBM Corp.). Descriptive statistics were used to present categorical variables such as frequency and percentage. Continuous variables were presented as mean (standard deviation/SD). The knowledge level about short stature was assessed using a continuous scale that was constructed based on participants' answers to 25- questions that

explored their knowledge about short stature (Table 2). Each correct answer was given a score of one, with a maximum attainable score of 25. The higher the score, the more knowledgeable the participant is. Independent sample t-test and ANOVA were used as appropriate to compare the mean knowledge score between different demographic groups. Binary logistic regression was conducted to identify factors that affect participants' knowledge about short stature. The dummy variable for the binary logistic regression utilized the mean knowledge score of the study participants (13.0) as the cut-off point. A p-value of 0<0.05 was set as the significant level.

Results

This study included a total of 6,852 individuals. The center area was home to nearly half (45.3%). More than half of the participants (65.4%) were between 18 and 30 years old. Females made up 54.7 % of the total participants. The average height of the participants in the study was 164.6 cm (10.2). More than half of the participants in the study (58.0%) reported they had a bachelor's degree. University students made up about 41.0 % of the study participants. Saudis made up the great majority of the participants (95.1%). Sixty-six percent of the study participants (60.6 %) were single.

Approximately half of the participants (55.0%) had heard of bone lengthening procedures. Around a quarter of the study participants (23.9%) were familiar with contemporary lengthening operations. The survey participants' primary source of information regarding the various types of contemporary

Table 1 Demographic Characteristics of Study Participants

Demographic Variable	Frequency (%)
Area of residency	
Northern area	697 (10.2%)
Southern area	943 (13.8%)
Central area	3,101 (45.3%)
Eastern area	695 (10.1%)
Western area	1,416 (20.7%)
Age (years)	
18–30	4,483 (65.4%)
31–40	956 (14.0%)
41–50	859 (12.5%)
51 and over	554 (8.1%)
Gender	
Female	3,745 (54.7)%
How tall are you? (Mean (sd) cm)	164.6 (10.2)
Education level	
Preparatory	149 (2.2%)
Secondary	1,690 (24.7%)
Diploma	592 (8.6%)
Bachelor	3,976 (58.0%)
Postgraduate	445 (6.5%)
Employment status	
University student	2,835 (41.4%)
Unemployed	1,344 (19.6%)
Retired	392 (5.7%)
Working in the healthcare field	670 (9.8%)
Working outside the healthcare field	1,611 (23.5%)
Nationality	
Saudi	6,515 (95.1%)
Marital status	
Single	4,155 (60.6%)
Married	2,439 (35.6%)
Divorced	187 (2.7%)
Widowed	71 (1.0%)
Have heard about bone lengthening operations	(n=5,833)
Yes	3,207 (55.0%)
Know the types of modern lengthening operations	(n= 5,833)
Yes	1,397 (23.9%)
Source of information of modern lengthening operations	(n=1,391)
Social media	644 (46.3%)
A doctor or healthcare professional	340 (24.4%)
Friends and Relatives	277 (19.9%)
Other sources	130 (9.3%)

Table 2 Perception About Height

Variable	Frequency (%)
Find height in relation to others	
Short	1,176 (17.2%)
Average	4,532 (66.1%)
Long	1,144 (16.7%)
Opinion of optimal height	
150–155 cm	
156–160 cm	
161–165 cm	
166–170 cm	
171–175 cm	
176–180 cm	
181 cm and over	
Know that there is a way to treat the problem of short stature	
Yes	2,771 (40.4%)
Satisfied with current height	
No	1,542 (22.5%)
Yes	5,310 (77.5%)
Extra length that want to get (n=1,542)	
less than 5 cm	47.2%
5-7 cm	34.7%
7-8 cm	6.9%
8-10 cm	6.1%
more than 10 cm	5.2%
The height is a social or occupational obstacle	(n= 6,704)
Yes	704 (10.5%)

lengthening operations was social media. More than half of the study participants (66.1%) reported that they find their height about others as average. More than half of the study participants (61.8%) believed that the optimal height is 150–170 cm. Most research participants (77.5 %) expressed satisfaction with their height. Nearly half of those unhappy with their height (47.2 %) said they wanted to gain less than 5 cm. A total of 40.4% of survey participants stated that they are aware of a treatment option for their small height. Only one-tenth of the research participants (10.5 %) said their height is a social or occupational barrier.

With a mean score of 13.0 (SD 5.8) out of 25, the participants demonstrated moderate knowledge of short stature (52.0 %). When participants were asked about the causes of short stature, genetic factors were the most commonly mentioned (79.2 %). Short stature can occur due to complications during pregnancy, which is the least usually reported cause (13.7 %). Malnutrition related to food quality (70.9 %) and the frequency of daily meals (60.9 %) may contribute to a problem in children's growth, contributing to short stature, according to more than half of the survey participants. Child abuse and neglect are variables that may contribute to a problem in a child's development, which may contribute to short stature, according to less than half of the study participants (47.3 %). Long-term usage of cortisone treatments for children is one of the variables that may contribute to a problem in children's growth, which may contribute to short stature, according to more than half of the study participants (58.1%).

Obesity, short, and flat faces were the most generally reported signs, if they appeared, for which the participants believed they should visit a doctor because they may be related to the problem of short stature. Radiographs and lab tests help diagnose the problem of short stature in children, according to 54.1 % and 66.2 % of study participants. Most study participants (86.0 %) agreed that the treatment strategy for short height varies depending on the leading cause of short stature. Most survey participants (71.9%) thought that taking certain types of nutritional supplements as prescribed by a doctor was an effective treatment for varying reasons of short stature. Surgical operations are one of the methods used to cure the problem of short stature, according to a similar percentage (69.1%). Hormonal therapy is one of the strategies used to treat the problem of short stature, according to 83.2 % of study participants.

A total of 76.6% of the study participants identified that hormonal therapy is suitable only for children before puberty for treating short-stature problems. Around one-quarter of the study participants (23.7%) identified that for surgical management. Nutritional balance, doing sports, and sufficient sleep were accurately identified as recommended practices to avoid the problem of short stature in children by 63.6%, 63.5%, and 56.1%, respectively.

Most survey participants (78.0%) stated that if they have a problem with short stature or want to enhance their own or their children's

Table 3 Knowledge About Short-Stature and Attitude Towards Treatment

Variables	Frequency (%)
Knowledge about short stature	
Causes	
Reason for short stature (you can choose more than one answer)?	
Short stature for genetic reasons	5,424 (79.2%)
Idiopathic short stature	2,118 (30.9%)
Short stature due to endocrine disorders	1,793 (26.2%)
Short stature due to problems during pregnancy	942 (13.7%)
Malnutrition related to the quality of food is one of the factors that may contribute to a problem in the growth of children, which may contribute to short stature	(n=6,469)
Yes	4,589 (70.9%)
Malnutrition related to the number of daily meals is one of the factors that may contribute to a problem in the growth of children, which may contribute to short stature	(n=6,469)
Yes	3,938 (60.9%)
Child abuse and neglect are factors that may contribute to a problem in the development of children, which may contribute to short stature	(n= 6,469)
Yes	3,060 (47.3%)
The long-term use of cortisone treatments for children is one of the factors that may contribute to a problem in the growth of children, which may contribute to short stature	(n=6,469)
Yes	3,760 (58.1%)
Clinical symptoms	
It is known that there are many diseases whose occurrence is associated with short stature in children. Signs, if appeared, I belief that they need to consult a doctor because they may be related to the problem of short stature (You can choose more than one answer)	
Obesity (% Yes)	4,214 (61.5%)
Short neck (% Yes)	3,991 (58.2%)
Flat face (% Yes)	2,767 (40.4%)
Tongue protrusion (% Yes)	2,642 (38.6%)
Small ears (% Yes)	2,431 (35.5%)
Face Rotation (% Yes)	2,051 (29.9%)
Methods of diagnosing the disease	
Diagnostic methods effective in diagnosing the problem of short stature in children (You can choose more than one answer)	
Radiograph (% Yes)	4,534 (66.2%)
Lab tests (% Yes)	3,705 (54.1%)
Methods of Treatment	
The method of treatment for the problem of short stature differs according to the main cause of short stature	(n=5,916)
Yes	5,090 (86.0%)

Table 3 (continued)

Variables	Frequency (%)
The use of some types of nutritional supplements - prescribed by the doctor -an effective treatment for some causes of the problem of short stature	
Yes	4,256 (71.9%)
Surgical operations is one of the methods used to treat the problem of short stature	(n=5,916)
Yes	4,088 (69.1%)
Hormonal therapy one of the methods used to treat the problem of short stature	(n=5,916)
Yes	4,920 (83.2%)
The following methods for treating the problem of short stature is suitable only for children before puberty	
Hormonal therapy	5,247 (76.6%)
Surgical management	1,624 (23.7%)
The following is recommended to avoid the problem of short stature in children (You can choose more than o\ answer)	
Nutritional balance	4,361 (63.6%)
Doing sports	4,349 (63.5%)
Proper sleep	3,844 (56.1%)
Attitudes	
I am ready to consult a doctor if I suffer from a problem of short stature or want to increase my height or the height of your children	(n=5,841)
Yes	4,556 (78.0%)
I am ready to give hormonal therapy to my children if they suffer from short stature problem to increase their height under medical supervision.	(n=5,841)
No	665 (11.4%)
Yes	2,357 (40.4%)
I don't have children	2,819 (48.3%)
I am ready to do surgical intervention if I suffer from a problem of short stature by lengthening the bones or want to increase my height.	(n=5,841)
Yes	2,484 (42.5%)
I am ready to have an operation or get treatment to get the height and stature I am looking forward to for me and my children	(n=5,820)
Yes	2,760 (47.4%)
Reasons for not agreeing to do operation or get treatment, you can choose more than one answer (n=4,092)	
Duration of treatment	1,501 (36.7%)
The cost	1,350 (33.0%)
The presence of an external device is annoying	1,339 (32.7%)
The work	763 (18.6%)
Embarrassment from family and relatives	436 (10.7%)
If an internal device is available that solves the problem of short stature with a higher cost for it, I am ready to perform the operation for me or my children	(n=5,769)
Yes	2,847 (49.3%)

Table 4 Knowledge Score Stratified by Demographic Characteristics

Demographic Variable	Mean Knowledge Score (SD)	P-value
Area of residency		
Northern area	13.8 (5.9)	≤0.001**
Southern area	13.3 (6.1)	
Central area	12.8 (5.8)	
Eastern area	13.7 (5.9)	
Western area	12.9 (5.4)	
Age (years)		
18–30	13.5 (5.9)	≤0.001**
31–40	12.6 (5.6)	
41–50	12.5 (5.2)	
51 and over	11.4 (5.8)	
Gender		
Male	13.3 (5.9)	0.013*
Female	12.9 (5.7)	
Education level		
Preparatory	10.2 (6.2)	≤0.001**
Secondary	12.6 (5.9)	
Diploma	12.0 (5.6)	
Bachelors	13.5 (5.7)	
Postgraduate	13.4 (5.7)	
Employment status		
University student	14.0 (5.9)	≤0.001**
Unemployed	11.5 (5.5)	
Retired	11.5 (6.0)	
Working in the healthcare field	15.3 (5.6)	
Working outside the healthcare field	12.2 (5.3)	
Nationality		
Non-Saudi	11.9 (5.6)	≤0.001**
Saudi	13.2 (5.8)	
Marital status		
Single	13.7 (5.8)	≤0.001**
Married	12.2 (5.7)	
Divorced	13.1 (5.6)	
Widowed	12.7 (5.3)	

*p≤0.05; **p≤0.001

height, they are willing to consult a doctor. Only 40.4% of research participants said they would give their children hormone therapy for short stature management under medical supervision if it proved to be a successful treatment for their condition. A similar amount (42.5%) of survey participants stated that they are willing to undergo surgical intervention - bone lengthening - if it is an effective way of managing short stature or if they desire to raise their own or their children's height. A total of 47.4 % of study participants said they are willing to have

Table 5 Factors Affecting Participants' Knowledge Identified by Binary Logistic Regression

Demographic Variable	Odds Ratio of Being Knowledgeable	95% Confidence Interval
Central area (Reference group)	1.00	
Southern area	1.07	(0.94–1.23)
Northern area	1.25	(1.07–1.47)**
Eastern area	1.21	(1.03–1.42)*
Western area	0.98	(0.90–1.12)
Age (years)		
18–30 (Reference group)	1.00	
31–40	0.81	(0.71–0.93)**
41–50	0.87	(0.76–1.00)*
51 and over	0.64	(0.54–0.76)***
Gender		
Female (Reference group)	1.00	
Male	1.01	(0.92–1.12)
Education level		
Preparatory (Reference group)	1.00	
Secondary	0.92	(0.83–1.02)
Diploma	0.70	(0.59–0.82)***
Bachelor	1.19	(1.10–1.28)***
Postgraduate	1.10	(0.91–1.34)
Employment status		
University student (Reference group)	1.00	
Unemployed	0.64	(0.57–0.71)***
Retired	0.64	(0.52–0.78)***
Working in the healthcare field	1.82	(1.53–2.16)***
Working outside the healthcare field	0.73	0.66–0.81)***
Nationality		
Saudi (Reference group)	1.00	
Non-Saudi	0.74	(0.60–0.92)**
Marital status		
Single (Reference group)	1.00	
Married	0.73	(0.66–0.79)***
Divorced	1.09	(0.81–1.46)
Widowed	0.74	(0.46–1.18)

*p≤0.05; **p≤0.01; ***p≤0.001

an operation or receive treatment to achieve the height and stature they desire for themselves or their children. With 36.7 %, 33.0 %, and 32.7 %, respectively, the most commonly reported barriers that prevented participants from being ready to perform surgery or receive treatment for short-stature problems were the length of treatment, the cost, and the intrusive presence of an external device. On the other hand, nearly half of the study participants (49.3%) said they would be willing to operate for themselves or their children if an internal device that solves the

problem of short stature at a higher cost became available.

Participants' knowledge scores about short stature differed significantly based on their area of residency, age, education level, employment status, nationality, and marital status ($p \leq 0.05$). Participants who were living in the northern and eastern area, aged 18–30 years, males, those with higher education (bachelor's degree or postgraduate), those who are working in the healthcare sector, Saudis, and single showed higher knowledge scores compared to others (Table 4).

Binary logistic regression confirmed that participants who are living in the northern and eastern areas, those who have bachelor's degrees, and those who are working in the healthcare field are more likely to be knowledgeable about short stature compared to others ($p \leq 0.01$) (Table 5).

Discussion

This study aimed to explore the knowledge about SS and attitudes towards its treatment among the general public in Saudi Arabia. The key findings of our research are: 1) around half of the study participants reported that they have heard about bone lengthening operations and around one-quarter the study participants reported that they know the types of modern lengthening operations, 2) social media was the main source of information of the study participants about the types of modern lengthening operations, 3) a total of 40.4% of the study participants reported that they know that there is a way to treat the problem of short stature, 4) the participants showed a moderate level of knowledge about short stature with the vast majority of the study participants identified that the method of treatment for the problem of short stature differs according to the main cause of short stature, 5) the majority of the study participants reported that if they suffer from a problem of short stature or want to increase their height or the height of their children, they are ready to consult a doctor regarding this problem, and only 40.4% of the study participants reported that they are ready to give their children hormonal therapy for the management of short stature under medical supervision if it was effective treatment for their condition, 6) a total of 47.4% of the study participants reported that they are ready to have an operation or get treatment to get the height and stature they are looking forward to for them or their children, 7) duration of treatment the cost, and the annoying presence of an external device were the most commonly reported barriers that prevented the participants from being ready to perform operation or get treatment for short stature problems.

Short stature can be subcategorized into familial, non-familial idiopathic short stature, and pubertal delay. Further sub classification can be according to short stature secondary to a small birth size (small for gestational age/ SGA) and systemic and endocrine diseases.[8] In this paper, participants showed a moderate level of knowledge about short stature with a mean score of 13.0 (SD 5.8) out of 25 (52.0%). Participants living in the northern and eastern areas, those with a bachelor's degree, and those working in the healthcare field are more likely to be knowledgeable about short stature than others ($p \leq 0.01$). Regarding the causes (79.2%) believed that short stature is linked to genetic or familial factors; this goes coherent with previous study that showed that the most common cause of SS was familial/genetic.[2] On the contrary, a study was done in China showing the primary cause of SS was idiopathic and endocrinal, while genetic causes were the least observed in this case.[9] However, the commonest cause of SS remains unclear as it varies and differs in each country. Regarding the impact of SS on individual life, our study showed that (22.5%) of participants are not satisfied with their height and would like to grow taller; this type of feeling can be linked to what was described in previous literature. A condition known as Height dysphoria or neurosis is a type of body image anxiety disorder in which the patient is unsatisfied or distressed because they perceive themselves as short.[10] Our study also showed that (10.5%) of participants reported that their height is a social or occupational obstacle. The correlation between height and life satisfaction was established in previous literature; the taller a person is, the more satisfied. Adults with short statures may experience unpleasant consequences throughout their lives. There are some evidence suggesting that the short-statured children and adults are socially and economically disadvantaged and their quality of life is also effected in compared to those with normal stature.[11] This obstacle of SS can be overcome with the modern development of medicine. There are various ways to treat SS, for instance, cosmetic or corrective lengthening surgeries or hormone replacements, and even physiotherapy.[2] Regarding knowledge of treatment methods, the vast majority of the study participants (86.0%) identified that the

treatment method for SS differs according to the leading cause. As for willingness, most of the study participants (78.0%) reported that if they suffer from a short stature problem or want to increase their height or their children's height, they are ready to consult a doctor regarding this problem. Furthermore, only 40.4% of the study participants reported that they would give their children hormonal therapy to manage short stature under medical supervision if it was an effective treatment. While 47.4% of the study participants reported that they are ready to have an operation or get treatment to get the height and stature they look forward to for themselves or their children. Duration of treatment, the cost, and the burden of the presence of an external device were the most commonly reported barriers that prevented the participants from being ready to perform the operation or get treatment for short stature problems with 36.7%, 33.0%, and 32.7%, respectively. Children with short stature might get distinctly diverse recommendations with varying levels of complexity and expense, as well as recommendations whose relative risks and benefits are unclear. Growing taller and reducing psychosocial disability while keeping positive risk/benefit and cost/benefit ratios are two justifications for treating childhood short stature.[12] Human-growth hormone-based hormone therapy for SS is pricey and costs rise as treatments are given for longer and at higher doses.[13] Low-dose androgen therapy using either injectable testosterone or oral oxandrolone (e.g. 1.25–2.5 mg/day) is one of the non-hGH growth-promoting treatments for short peri-pubertal boys. Although neither is FDA-approved for growth acceleration, both have been shown in controlled studies to accelerate growth by 3-5 cm/year for 1-3 years.

Short-statured children and adolescents have more internalizing issues and suffer from height-related quality of life deficits, which adds to caregiver stress and lowers parents' quality of life.[14] Due to their condition, short-statured children experience severe social, academic, and psychological challenges.[5] These include stigmatization and discrimination, low self-esteem, body image issues, a lack of developmentally appropriate social skills, and social withdrawal, increasing the parents' responsibilities and concerns. Multidisciplinary interventions in the context of pediatric short stature should focus on the children's or adolescents' psychosocial functioning in addition to growth hormone therapy. They should also give the parents cognitive and behavioral management techniques to deal with their child's physical, emotional, social, and behavioral issues.[14] In order to improve the results of family-centered psychosocial therapies aiming at fostering parents' adaptation, caregiving stress should be consistently assessed and chosen as a strategic intervention target.

The nature of the cross-sectional survey design is itself causing a limitation as it limits the ability to identify causality between study variables. Furthermore, more studies are needed to assess knowledge about SS and attitudes toward its treatment among the general public using similar survey tools; different studies used different tools to explore participants' knowledge, which limited our ability to compare our findings. In this study, we employed a quantitative methodology with pre-set responses, which might not have allowed participants' views to provide varied but valuable qualitative information. As a result, our findings must be interpreted carefully.

In conclusion, Saudi Arabians have a moderate understanding of SS, which needs to be improved. Campaigns to increase the general public's and parents' knowledge about SS, which is ultimately connected to earlier diagnosis and better management outcomes. Additional research is required to examine the most effective strategies for raising public knowledge of SS.

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RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v55n3.2990
Majalah Kedokteran Bandung. 2023;55(3):142–148

Majalah Kedokteran Bandung (MKB)

Received: August 28 2022
Accepted: March 27, 2023
Available online: September 30, 2023

Oxygen Saturation Diagnostic Accuracy Against COVID-19 in Rural Areas of Indonesia

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Abstract

As a country with a high proportion of rural areas, Indonesia continues to struggle with a rapid and accurate diagnosis of COVID-19, necessitating the development of a diagnostic tool or parameter that is less expensive, easier to obtain, and produces rapid results. This retrospective study aimed to explore the diagnostic accuracy of oxygen saturation in detecting COVID-19 in rural areas of Indonesia. Data were collected consecutively from medical records of adult patient (30–90 years old) suspected of having COVID-19 based on the WHO criteria and underwent RT-PCR swab test in three (3) hospitals in one of the regions of Indonesia during the timeframe of May 1, 2020 to September 31, 2021. Analysis was conducted using the cross-table analysis with sensitivity, specificity, positive predictive value, negative predictive value, and area under the curve (AUC) as the variables with their respective confidence interval. Results indicated that 548 of 700 patients included in the analysis were confirmed positive for COVID-19 based on the RT-PCR test results. The sensitivity, specificity, positive predictive value, negative predictive value, and area under the curve (AUC) value of oxygen saturation for detecting COVID-19 were 33% (CI 95% 29–37%), 78% (CI 95% 72–85%), 84% (CI 95% 80–89%), 24% (CI 95% 21–28%), and 56% (CI 95% 51–61%), respectively. Thus, the oxygen saturation level alone does not have adequate diagnostic accuracy for the diagnosis of COVID-19 and, therefore, is not recommended to be used for diagnosing COVID-19.

Keywords: COVID-19, Indonesia, oxygen saturation

Introduction

China reported a case of pneumonia caused by infection with a novel coronavirus on December 31, 2019. The *World Health Organization* (WHO) named the new virus as a *Severe Acute Respiratory Syndrome Coronavirus 2* (SARS-CoV2) and the disease *Coronavirus Disease* 2019 (COVID-19) on February 11, 2020. WHO declared COVID-19 as a pandemic on March 11, 2020.[1] WHO reported 228,807,361 confirmed cases of COVID-19 worldwide until the end of September 2021, with a death toll of 4,697,099 cases, and this number was still growing at the time this research was conducted.[2] In addition to the increase in mortality and morbidity, the COVID-19 pandemic has significantly impacted almost every country's public health and social aspects.[3]

Until now, WHO still recommends the use of molecular detection methods/Nucleid Acid Amplification Test (NAAT) such as the Real Time Polymerase Chain Reaction (RT-PCR) examination or the SARS-CoV2 Rapid Antigen Diagnostic Test (RDT) as a tool to establish the diagnosis of COVID 19.[4] Several results from the systematic review and meta-analysis that have been carried out also show fairly good diagnostic accuracy of the two tests.[5,6] Despite their high diagnostic accuracy, both tests have limitations. The RT-PCR test is a costly, labor intensive, and difficult examination to provide in rural areas. Therefore, the RDT antigen test has a lower sensitivity and is not recommended for use after day 7 of symptom onset or in individuals with low viral loads.[7] While a complete blood count has a relative weakness in terms of diagnostic accuracy, a chest X-ray has a significant subjectivity.

Indonesia, an archipelagic country with a significant disparity in resources and the quality of health services between the city center and its

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regions, continues to face difficulties in making a rapid and accurate diagnosis of COVID-19, particularly in underdeveloped areas that are remote from the city center and generally lack health services.[8] As a result, a diagnostic tool or parameter is required that is less expensive, does not require special skills, produces rapid results, and is easily accessible even in the least developed areas. Oxygen saturation is one of them. Until this research was conducted, the researchers found that oxygen saturation can predict the mortality and need for a mechanical ventilation procedure (invasive or non-invasive) in COVID-19 patients in the hospital.[9,10] But, research still needs to be done to determine its diagnostic accuracy in COVID-19. Because of those reasons, the researchers want to know the diagnostic accuracy of this index.

The diagnostic research design to be carried out uses a single-test approach, which is not the best hierarchical diagnostic research design. This is because a research design like this needs to follow everyday practice. Therefore, the appropriate approach is multivariable.[11] However, the design of this study was still carried out because first, the researcher wanted to conduct a more in-depth study of oxygen saturation alone, without paying attention to information from other indices, which, in the researcher's opinion, have relative weaknesses in diagnosing COVID-19. Second, this study is an initial study where researchers want to know whether oxygen saturation has the potential to be developed as a diagnostic tool. The research can be continued with a more ideal research design if it has potential.

The study's primary objective was to ascertain the diagnostic accuracy of oxygen saturation in the detection of COVID-19 (in the form of sensitivity, specificity, predictive value, and area under the curve). Additionally, the diagnostic accuracy of oxygen saturation in detecting COVID-19 at an onset of more than 7 days and during a COVID-19 case outbreak in Indonesia is being determined. The researchers hypothesize that oxygen saturation also has a high diagnostic accuracy for COVID-19 detection and thus has the potential to be used in COVID-19 diagnosis.

Methods

The study was conducted from October to November 2021 by collecting patient data from the medical records of patients suspected of having COVID-19 and agreeing to carry out RT-PCR examinations at three hospitals carrying out COVID-19 treatment in North Luwu Regency, South Sulawesi Province, Indonesia, from May 1, 2020, to September 31, 2021. The research was approved by the Health Research Ethics Committee, Faculty of Medicine and Health Sciences, University of Muhammadiyah Makassar, Indonesia, with the number 001/UM.PKE/X/43/2021.

Patients who met the inclusion criteria were adults (30–90 years) suspected of having COVID-19 based on WHO criteria[5] and agreed to carry out a diagnostic test using RT-PCR. The specific age range was chosen due to their increased susceptibility to low oxygen saturation levels during COVID-19 infection. Researchers excluded patients confirmed positive for COVID-19 by the RT-PCR method within three months before coming to the hospital.

All patients who met the inclusion criteria based on the screening and evaluation results of medical records by the first investigator (M.A.M) and the fourth investigator (A.S.) were consecutively included in this study. Oxygen saturation levels are measured by placing a pulse oximeter that has been through internal calibration on one of the patient's fingers for a few seconds. For RT PCR, the examination is carried out using the nasopharyngeal and oropharyngeal swab method based on the Ministry of Health of the Republic of Indonesia guidelines. Both the oxygen saturation and RT-PCR results were taken from the medical records.

The researcher used RT-PCR as the gold standard test, with positive or negative results,[4] and the oxygen saturation threshold of 90% to determine whether the patient was hypoxemic. An oxygen saturation of 95–100% is considered normal, while values under 90% are significantly associated with progressive deterioration, followed by increased mortality risk for those with saturation below 70%. Symptoms of deterioration include increased respiratory rate, pulse rate, and low blood pressure, commonly considered hallmark symptoms of hypoxemia.[12] The nasopharyngeal and oropharyngeal swab samples were sent to the Central Health Laboratory in Makassar (approximately 8-10 hours by car from the research site), one of the largest reference laboratories appointed by the Indonesian government to assess the RT-PCR examination results. The assessment is conducted by a trained and certified microbiologist unaware of the patient's oxygen saturation examination results.

The researcher obtains the patient's oxygen

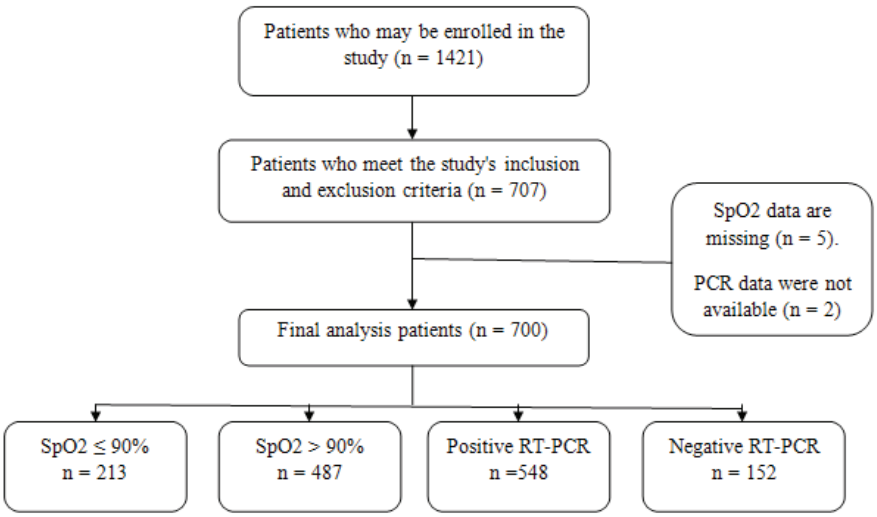

Figure 1 Research Flowchart

saturation data from the oxygen saturation data obtained the first time the patient arrives at the health facility without using oxygen (room air); if the oxygen saturation data obtained in all medical records is oxygen saturation with the assistance of oxygen therapy, the researcher elects to obtain the lowest oxygen saturation level of the patient. Suppose no patient oxygen saturation data is available before the RT-PCR swab. In that case, the researcher obtains oxygen saturation data following the RT-PCR swab with the closest time interval.

Additionally, researchers collected data on the subject's characteristics, including age, gender, comorbidities, and the severity of the patient's disease. Additionally, the researchers examined the diagnostic accuracy of oxygen saturation at disease onset after the seventh day and during an outbreak in Indonesia. The data was analyzed using IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp.) in the form of a cross-table analysis with research outcomes in the form of sensitivity, specificity, positive predictive value, negative predictive value, and area under the curve (AUC). Each has a confidence interval associated with it.

With an expected sensitivity of 85%, a generalization error of 5%, a precision of 3%, and a prevalence of COVID-19 of 73 percent in patients suspected of having COVID-19 based on literature, to determine the sensitivity of oxygen saturation, 745 patients with suspected COVID-19 are required. According to the literature, the prevalence of non-COVID-19 in patients with suspected COVID-19 is 27%. With an expected specificity of 85%, a generalization error of 5%, and a precision of 5%, 726 patients with suspected COVID-19 are required. Additionally, researchers are interested in the area under the curve (AUC) of oxygen saturation to diagnose COVID-19. According to the literature, COVID-19 is present in 73% of patients with suspected COVID-19. With an expected AUC of 80%, a precision of 10%, and an alpha of 5%, this study required 118 patients with suspected COVID-19 infection, with an expected composition of 32 positive and 86 negative patients.

Results

The RT-PCR swab results were not indeterminate. This study had five missing data points for oxygen saturation results and two for RT-PCR results. The researcher chose to omit specific data from the missing data set. Due to the scarcity of missing data discovered, namely 7 out of 700 samples (0.01 percent), data exclusion did not result in bias, though it did reduce research power. The patient characteristics are listed in Table 1.

Between oxygen saturation examination and RT-PCR swab sampling, the median time was 1 day (range, 0–4 days). The diagnostic accuracy of oxygen saturation in diagnosing COVID-19 is shown in Table 2. The RT-PCR examination

Table 1 Characteristics of Patients (n=700)

Variables	Description
Age (years) *	55 (30–89)##
Gender**	
Male	320 (45.7%)
Female	380 (54.3%)
Comorbid#	
Hypertension	136 (19.4%)
Diabetes mellitus	134 (19.1%)
Stroke	5 (0.7%)
Coronary artery disease	13 (1.9%)
Congestive heart failure	12 (1.7%)
COPD	0 (0.0%)
Asthma	11 (1.6%)
Pulmonary tuberculosis	14 (2.0%)
Additional diseases	34 (4.9%)
The severity of the disease	
Severe	216 (30.9%)
Mild	484 (69.1%)

*Numeric variables with abnormal data distribution are presented in the medium (minimum–maximum); **Categorical variables are expressed as n (percent); #Comorbidity type indicates only patients who have comorbidities in the form of n (percent); ##There are three (0.4%) missing values for the patient's age

identified 548 confirmed COVID-19 patients. The sensitivity and specificity of oxygen saturation for detecting COVID-19 were 33% (CI 95% 29–37%) and 78% (CI 95% 72–85%), respectively, with 84% positive predictive values (CI 95% 80–89%), and 24% negative predictive value (CI 95% 21–28%). Apart from mild discomfort, there were no serious adverse effects associated with the RT-PCR examination performed on the patient.

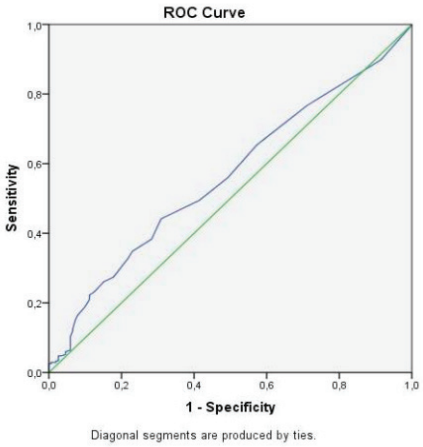

Figure 2 Oxygen Saturation ROC Curve for the Diagnosis of COVID-19

The *Receiver Operating Characteristic* (ROC) curve for oxygen saturation for the diagnosis of COVID-19 is shown in Figure 2, with an AUC value of 56% (CI 95% 51–61%) for oxygen saturation.

The sensitivity and specificity of oxygen saturation values for diagnosing COVID-19 are calculated in Table 3 using a variety of alternative intersection points. At 90.50, oxygen saturation has a sensitivity of 32% and a specificity of 21%,

Table 2 Cross Tabulation of Diagnostic Accuracy of Oxygen Saturation in COVID-19

		RT PCR		
		Positive (n=548)	Negative (n=152)	Total (n=700)
Oxygen Saturation	Hypoxemia	180	33	213
	Non-Hypoxemia	368	119	487

Table 3 Oxygen Saturation Sensitivity and Specificity Values For COVID-19 Diagnosis Using Various Cut-Off Points

Cut-Off Points	Sensitivity	-1 Specificity	Specificity
≤98.50	0.89	0.91	0.09
≤97.50	0.76	0.71	0.29
≤95.50	0.56	0.49	0.51
≤92.50	0.38	0.28	0.72
≤90.50	0.32	0.21	0.79
≤85.50	0.21	0.11	0.89
≤80.50	0.12	0.06	0.94
≤70.50	0.06	0.04	0.96
≤60.50	0.03	0.02	0.98
≤51.50	0.02	0.007	0.993

Table 4 Cross Tabulation of Diagnostic Accuracy of Oxygen Saturation For COVID-19 With Onset Greater Than 7 days

		RT PCR		Total (n=57)
		Positive (n=42)	Negative (n=15)	
Oxygen Saturation	Hypoxemia	15	1	16
	Non-Hypoxemia	27	14	41

Sensitivity (95% CI)=36% (21–50%); Specificity (95% CI)=93% (81–106%); Positive predictive value (95% CI)=94% (82–106%); Negative predictive value (95% CI)=34% (20–49%)

while at 80.50, it has a sensitivity of 12% and a specificity of 94%.

The diagnostic accuracy of oxygen saturation is determined in Tables 4 and 5 for the diagnosis of COVID-19 with an onset date greater than the seventh day and during a case outbreak in Indonesia, respectively.

Discussion

The results of data analysis in Table 1 indicate that oxygen saturation as a single test for COVID-19 has a low diagnostic accuracy. Thus, while oxygen saturation level is generally not recommended as a screening tool for COVID-19, it may be used to assist clinicians in the final stages of diagnosis due to their relatively high specificity.

While the diagnostic accuracy is low, the researchers discovered in Table 2 that the lower the oxygen saturation level, the lower the sensitivity, but the higher the specificity, for diagnosing COVID-19. Additionally, a sufficient oxygen saturation PPV (84 percent) can assist a clinician in initiating or performing emergency mitigation of patients suspected of having COVID-19 in certain circumstances, such as when the results of the reference examination take a long time to confirm the diagnosis, or when the clinician has a strong suspicion that the patient has COVID-19 but other index tests cannot detect it (onset after day 7, lack of viral load, technical error of sampling). Of course, this benefits clinicians or health workers working in rural areas of Indonesia with limited resources, especially where the speed and accuracy of diagnosis could be better.

We also examined the diagnostic accuracy of oxygen saturation at the onset above day 7 in diagnosing COVID-19 as a secondary outcome. This is based on observations made in the research area that patients suspected of COVID-19 may delay hospitalization for a variety of reasons. Some were admitted to the hospital with an onset date greater than the seventh day, and some with an onset date greater than the fourteenth day. In these instances, the majority of rapid tests for SARS-CoV-2 antigen were negative. This could be due to the rapid antigen test's decreased sensitivity on day 7 of onset. Interestingly, the investigators discovered that the oxygen saturation specificity was 93 percent and the PPV was 94 percent at onset after day 7. See Table 4.

Although these results are based on a small sample size and are not primary outcomes, they can serve as a starting point for further research into whether oxygen saturation can be used as a good diagnostic tool to diagnose COVID-19 after the 7th day of onset in several cases where the rapid antigen test results are negative and the RT-PCR examination results are unavailable due to a variety of factors. Additionally, in areas with limited access to rapid diagnostic testing, oxygen saturation can be used to assist clinicians

Table 5 Cross tabulation of Diagnostic Accuracy Of Oxygen Saturation for COVID-19 during a Spike in COVID-19 Cases

		RT PCR		Total (n=535)
		Positive (n=396)	Negative (n=139)	
Oxygen Saturation	Hypoxemia	125	31	156
	Non-Hypoxemia	271	108	379

Sensitivity (95% CI)=32% (27–36%); Specificity (95% CI)=78% (71–85%); Positive predictive value (95% CI)=80% (74–86%); Negative predictive value (95% CI)=28% (24–33%)

and other health care providers in mitigating risks, such as the placement of treatment rooms in hospitals, administering therapy to high-risk groups, and contact tracing), and burial protocols for suspected COVID-19 patients.

Additionally, we analyzed the diagnostic accuracy of oxygen saturation for the first and second spikes in cases in Indonesia (1 December 2020–28 February 2021) and 1 June–30 September 2021, respectively. This is based on clinicians' reservations about using oxygen saturation levels in Indonesia, where the number of cases is quite low. The sloping number of cases suggests a low transmission rate and the possibility of detecting COVID-19 cases. The researchers conducted this analysis to avoid overtreatment or undertreatment. According to Table 4, the diagnostic accuracy of oxygen saturation during the peak of cases in Indonesia is lower and comparable to the diagnostic accuracy of the cumulative oxygen saturation.

The study's limitations include the fact that the reference examination was not conducted according to standard procedures, and that the RT-PCR sampling, which should be performed twice within a 24-hour interval, was performed only once due to resource constraints in the area where the study was conducted. Thus, if the first RT-PCR swab yields a negative result, the second swab cannot be confirmed. This increases the likelihood of false negatives. Then, in some samples, oxygen saturation was determined while the patient was receiving oxygen therapy, resulting in an oxygen saturation level that did not reflect reality. Additionally, diagnostic research should ideally employ a multivariable test approach, rather than with a single test method. Diagnostic modalities such as lymphocyte count, neutrophile lymphocyte ratio (NLR), chest X-ray, and chest CT could be considered in addition to oxygen saturation in diagnosing COVID-19. This is also why the diagnostic accuracy of the index studied may be suboptimal.

The advantages are that this study employs a cross-sectional design, which is the optimal design for diagnostic research; the oxygen saturation index used in this study is a non-invasive medical device that is inexpensive, easy to access and use; and thus, further research is simple to conduct and apply to health services in the underdeveloped area.

As far as researchers are aware, there has been no published research on the diagnostic accuracy of oxygen saturation for COVID-19 in Indonesia or elsewhere until now, so the researchers hope that the findings of this study can serve as input and a foundation for future research.

In conclusion, due to the low diagnostic accuracy of oxygen saturation levels as a single test, they are not recommended for the diagnosis of COVID-19. Further research should be conducted to determine the diagnostic accuracy of oxygen saturation using a multivariable test approach and a larger sample size in a larger health center.

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