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Original Research Report

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Original Research Report

CLINICAL PROFILES AND IL-6 LEVEL ANALYSIS OF CRITICAL COVID-19 PATIENTS RECEIVING LOPINAVIR-RITONAVIR

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ABSTRACT

The COVID-19 pandemic has affected over 760 million individuals worldwide, resulting in more than 6.8 million reported deaths. Early detection of patient deterioration can assist in predicting outcomes and prioritizing healthcare services based on evidence-based indicators. Interleukin-6 (IL-6) is a cytokine that plays a role in the inflammatory process, making it a potential parameter for assessing a patient's inflammatory state. This study aimed to analyze the characteristics, laboratory profiles, and IL-6 levels of COVID-19 patients. This study used a retrospective cohort study design with medical record data. The characteristics (n=68) and IL-6 levels (n=52) of the patients on the first, third, and sixth days of treatment were recorded consecutively. The mean age of the patients was 49 years, with the majority being male (72%) and the most prevalent comorbidity being hypertension (29%). The average duration of hospitalization was 10.94 days. Shortness of breath was the most commonly reported symptom (45.6%). The medians of neutrophil-lymphocyte ratio, C-reactive protein, procalcitonin, ferritin, and D-dimer were above normal. Significant differences were observed in lymphocytes (p=0.046), procalcitonin (p=0.023), and D-dimer (p=0.000) between survivor and non-survivor patients. Significant dynamic changes in IL-6 levels were observed from the first day to the sixth day (p=0.014) and from the third day to the sixth day (p=0.041). In conclusion, risk stratification, laboratory profiles, and IL-6 levels play a role in assessing the severity and outcomes of COVID-19 patients.

Keywords: COVID-19; pandemic; IL-6; clinical characteristics; human and health

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Highlights:
1. Lymphocyte, procalcitonin, D-dimer, and high IL-6 levels in COVID-19 patients are associated with a poor prognosis.
2. IL-6 serial measurement for COVID-19 patients may be a potential indicator for evaluating the severity and patient outcomes.

INTRODUCTION

Over 760 million people have been infected with COVID-19. The pandemic has reported more than 6.8 million deaths. The number of new COVID-19 cases has started to decrease globally. However, in several regions the numbers continued to exceed one million patients every month, with 28,000 patients dying (World Health Organization 2022). The case fatality rate caused by the SARS-CoV-2 virus (3–4%) was lower than those of the Middle East respiratory syndrome (MERS) (34%) and severe acute respiratory syndrome (SARS) (11%). However, the total number of deaths from this virus has far exceeded the mortality rates of MERS and SARS (Park et al. 2020). Data from Southeast Asian countries revealed that Indonesia's case fatality rate remained over 1% in December 2022 (Minister of Health of the Republic of Indonesia 2023). Administering lopinavir-ritonavir resulted in a

shorter length of stay in the intensive care unit (ICU), with a median of six days compared to eleven days for the standard care group. This is very important in a pandemic because of the limited capacity of the ICU (Owa & Owa 2020).

Clinical manifestations of COVID-19 can appear on a wide spectrum, from asymptomatic to critical symptoms. The critical symptoms are characterized by acute respiratory distress syndrome, which requires mechanical ventilation, and septic shock (World Health Organization 2022). Various studies have reported that the pathophysiology of COVID-19 is closely related to acute inflammation. Interleukin-6 (IL-6) is the main proinflammatory cytokine involved in controlling cell differentiation, migration, proliferation, and apoptosis (Rehman et al. 2017).

Several studies have revealed a strong correlation between serum IL-6 levels and impending respiratory failure (Coomes & Haghbayan 2020). In addition, serum SARS-CoV-2 nucleic acid (RNAaemia), which is highly associated with cytokine storms, is closely correlated with very high serum levels of IL-6 (Chen et al. 2020). As IL-6 is a key factor to indicate the degree of inflammation, evaluating the dynamic changes in IL-6 levels is important for identifying disease progression and predicting cytokine storms. Therefore, this study aimed to assess the IL-6 levels of critical COVID-19 patients receiving lopinavir-ritonavir therapy.

MATERIALS AND METHODS

This study was a descriptive observational study with a cohort-retrospective study design. This study utilized the medical records of patients with positive polymerase chain reaction (PCR) results for COVID-19 as the study sample. The inclusion criteria were patients aged >18 years with critical symptoms who were hospitalized and received lopinavir-ritonavir therapy in the COVID-19 Isolation Room of the Intensive Care Unit at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, in the period of June–October 2020. Lopinavir-ritonavir therapy was an antiviral therapy that was used at the beginning of the pandemic, according to the World Health Organization (WHO) guidelines (The Indonesian Society of Respirology 2020). The sampling technique used in this study was total sampling, with a total sample of 68.

Data collected included the patient's characteristics, the laboratory profiles that were examined on the first day of treatment, and IL-6 levels on the first, third, and sixth days of treatment. All data were statistically analyzed using IBM SPSS Statistics for Windows Version 20.0 (IBM Corp., Armonk, N.Y., USA). The Kolmogorov-Smirnov test was then applied to determine the homogeneity of the data. Data on hemoglobin and hematocrit that were normally distributed were analyzed using the independent t-test, while the other variables were analyzed using the Wilcoxon test. A p-value of <0.05 was considered statistically significant (Poudel et al. 2021). The tables and narratives in this article are used to present the research findings descriptively.

This study was approved by the Health Research Ethics Committee at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, with a reference number 149/113/Komitlitkes/VII/2021 dated 15/7/2023.

RESULTS

The number of PCR-positive COVID-19 patients aged 18–65 years who received lopinavir-ritonavir therapy in the Intensive Care Unit was 68 patients. However, only 52 patients had complete data on their IL-6 levels. Table 1 shows the COVID-19 patients' characteristics. The patients were 49 (±10.81) years old on average and mostly men (72%). The most common comorbidity was hypertension (29%), while the average length of stay was 10.94±8.1 days. Shortness of breath (45.6%) was the most prevalent clinical manifestation among the patients.

Table 1. The COVID-19 patients' characteristics.

Variables	n (%)
Age (years)	
Mean±SD	49.04 (±10.81)
Median (min-max)	52.5 (24-65)
Sex	
Male	49 (72.1)
Female	19 (27.9)
Comorbid	
Hypertension	20 (29.4)
Diabetes mellitus	19 (27.9)
Obesity	18 (26.4)
Coronary heart disease	4 (5.8)
Asthma	4 (5.8)
None	26 (38.2)
Length of stay (days)	
Mean±SD	10.94±8.1
Median (min-max)	9 (6-54)
Clinical manifestation	
Shortness of breath	31 (45.6)
Dry cough	10 (14.7)
Fever	7 (10.3)
Anosmia	6 (8.8)
Diarrhea	6 (88)
Sore throat	3 (4.4)
Myalgia	3 (4.4)
Loss of consciousness	2 (2.9)

Table 2. Laboratory profiles of the COVID-19 patients.

Variables	Total (n=68)	Survivor (n=30)	Nonsurvivor (n=38)	p
Hemoglobin (g/dL)				0.239
Median (min-max)	13.52 (9.88-17.3)	13.2 (9.88-17.02)	13.65 (10.26-17.3)	
Mean±SD	13.48±1.69	13.21±1.75	13.69±1.64	
Hematocrit (%)				0.160
Median (min-max)	38.9 (27.89-52.2)	38.05 (27.89-50)	39.4 (30.6-52.2)	
Mean±SD	39.49±5.32	38.47±5.01	40.3±5.48	
Leukocyte ($x10^9$/L)				0.236
Median (min-max)	7.57 (1.96-38.89)	7.05 (3.72-20.3)	8.4 (1.96-38.89)	
Mean±SD	8.85±5.78	7.82±3.46	9.66±7.04	
Neutrophil ($x10^9$/L)				0.562
Median (min-max)	10.49 (4.3-22.7)	9.92 (5.42-22.7)	10.56 (4.3-19.12)	
Mean±SD	11.04±3.69	10.75±4.02	11.27±3.45	
Lymphocyte ($x10^9$/L)				0.046
Median (min-max)	1.05 (0.19-2.94)	1.24 (0.59-2.7)	1 (0.19-2.94)	
Mean±SD	1.19±0.61	1.32±0.51	1.1±0.67	
NLR				0.097
Median (min-max)	6.82 (1.72-49.26)	5.7 (2.14-24.76)	8.95 (1.72-49.26)	
Mean±SD	9.61±8.47	7.01 ± 4.87	11.67±10.07	
Thrombocytes ($x10^9$/L)				0.277
Median (min-max)	246.5 (96-673)	272.5 (122-412)	242 (96-673)	
Mean±SD	258.4 ± 96.27	267.9±85.39	250.89±104.57	
Procalcitonin (ng/mL)				0.023
Median (min-max)	0.24 (0.01-70.41)	0.15 (0.01-6.63)	0.32 (0.06-70.41)	
Mean±SD	1.78±8.62	0.5±1.21	2.8±11.45	
CRP (mg/L)				0.058
Median (min-max)	10.25 (0.6-89)	7.15 (1.1-27.4)	12 (0.6-89)	
Mean±SD	12.6±12.96	9.18±7.31	15.3±15.66	
Ferritin (mcg/L)				0.394
Median (min-max)	1187 (38.5-7340.5)	1161.1 (38.5-4361)	1241.85 (71.73-7340.5)	
Mean±SD	1460.84±1254.24	1299.91±1017.6	1587.89±1414.01	
D-dimer (ng/mL)				0.000
Median (min-max)	1495 (310-9280)	955 (380-6340)	1950 (310-9280)	
Mean±SD	1991.59±1706.83	1316.6±1168.73	2524.47±1882.48	

Table 2 demonstrates the patients' laboratory characteristics on the first day of treatment. The medians for the neutrophil-lymphocyte ratio (NRL), procalcitonin, C-reactive protein (CRP), ferritin, and D-dimer were found to be higher than the normal range. These were even higher in nonsurvivor patients. The statistical tests revealed significant differences in lymphocytes (p=0.046), procalcitonin (p=0.023), and D-dimer (p=0.000) among the survivor and nonsurvivor patients.

Table 3 shows the COVID-19 patients' IL-6 profiles. The mean IL-6 levels during the treatment were 256.42±475.99 on the first day, 224.97±515.49 on the third day, and 172.32±338.79 on the sixth day. The nonsurvivor group showed higher IL-6 levels than the survivor group. There was a significant difference in IL-6 levels on the third day (p=0.027) and sixth day (p=0.003), according to the statistical tests. The non-parametric Wilcoxon signed-rank test results indicated dynamic changes in IL-6 levels. There were significant changes in IL-6 levels from day 1 to day 6 (p=0.014) and from day 3 to day 6 (p=0.041), while there was no significant difference from day 1 to day 3 (p=0.321).

DISCUSSION

In this study, men were more infected with the COVID-19 virus and fell into critical condition more

Table 3. IL-6 level profiles of the COVID-19 patients.

IL-6 variable (pg/mL)	Total (n=52)	Survivor (n=26)	Nonsurvivor (n=26)	p
Day 1				
Median (min-max)	95.08 (1.77-3029.17)	79.41 (1.77-1254.69)	99.84 (52.95-3029.17)	0.062
Mean±SD	256.42±475.99	185.76±272.00	327.07±614.67	
Day 3				
Median (min-max)	113.79 (2.50-3651.89)	89.48 (2.50-486.63)	146.48 (20.57-3651.89)	0.027
Mean±SD	224.97±515.49	119.44±131.21	330.50±708.31	
Day 6				
Median (min-max)	91.61 (1.51-2187.50)	35.73 (1.51-705.61)	126.20 (3.1-2187.5)	0.003
Mean±SD	172.32±338.79	82.41±138.53	262.22±445.14	

often than women. Men also had higher levels of angiotensin-converting enzyme-2 (ACE2) expression and activity due to several factors (Liu et al. 2020a). ACE2 is found in many organs, such as the upper respiratory tract, the lungs, the heart, the kidneys, and blood vessel linings. Its presence in the testis is very high. Furthermore, estrogen has been shown to protect women from COVID-19 (Acheampong et al. 2020, Sama et al. 2020, Wulandari et al. 2021).

Patients with comorbidities are more likely to develop critical conditions. These results are supported by other studies, which reported that cardiovascular disease, diabetes, and hypertension increase the risk of critical conditions and require intensive care (Honardoost et al. 2021). Chronic diseases (such as cardiovascular disease, hypertension, and diabetes) are associated with the pathogenesis of COVID-19. These diseases can circulate proinflammatory cytokines, weaken the innate immune response, and enhance viral entrance into cells because both ACE2 and viruses require glucose to perform their activities. In addition, metabolic disorders can lower immune function by interfering with macrophage and lymphocyte functions, making individuals more susceptible to infection and falling into a critical condition (Badawi & Ryoo 2016, Kusuma & Ardiany 2021).

This study showed that the critical COVID-19 patients' average length of stay in the intensive care unit was 10.9 days. These results were better when compared to a study in the Netherlands, where the patients' average length of stay in the intensive care unit was 20.6 days during the first wave of the pandemic (February–May 2020). However, the Netherlands study showed a decrease to 17.2 days during the second wave of the pandemic (October 2020–January 2021) and 16 days during the third wave of the pandemic (February–June 2021) (Dongelmans et al. 2022).

Shortness of breath and dry cough were the most common clinical manifestations in this study. These results agreed with a study in China, which reported that 43% of critical COVID-19 patients complained of cough, sore throat, and shortness of breath (Ding et al. 2021). Coughing is caused by neurotropism (direct invasion of SARS-CoV-2 via ACE2 receptors), neuro-inflammation, and neuro-immunomodulation through the vagal sensory nerves (Song et al. 2021). The SARS-CoV-2 virus enters through the respiratory tract. Therefore, clinical manifestations in the upper and lower respiratory tracts are often found. It is not uncommon to find acute respiratory distress syndrome, which includes critical COVID-19 (Attaway et al. 2021).

Most of the critical COVID-19 patients showed lymphopenia, which is consistent with viral infection conditions (Zhong et al. 2021). A study reported that coronavirus infection induces lymphocyte clearance and inhibits immune function (Xu et al. 2020). This function acts as a potential immunological mechanism for disease progression. Apart from lymphopenia, critical COVID-19 patients also experience increased levels of inflammatory parameters (such as C-reactive protein, procalcitonin, ferritin, and D-dimer). Increased serum C-reactive protein, driven by IL-6 and other inflammatory cytokines, can be biomarkers for severe manifestations of COVID-19 because the patients may experience cytokine storms. This infection causes the activation of monocytes, macrophages, and dendritic cells (Melo et al. 2021). Other studies reported that increased procalcitonin was closely related to COVID-19 severity. This increase may be related to bacterial co-infection, which is mediated by an upregulation of tumor necrosis factor-alpha (TNF-α) and IL-6 (Hu et al. 2020). In addition, hyperinflammation occurs in COVID-19, causing a dysregulated immune response that may also trigger the production of procalcitonin (Tong et al. 2022). D-dimer is the most sensitive coagulation parameter in COVID-19 and indicates the risk of thrombosis (Conte et al. 2021). D-dimer levels are commonly elevated in elderly patients with comorbidities. The presence of viremia and cytokine storm syndrome in COVID-19 indicates a spike in pro-inflammatory cytokines that are uncontrollable by anti-inflammatory agents, thus overwhelming the coagulation cascade. Additionally, hypoxia predisposes to thrombosis through numerous signaling pathways (Poudel et al. 2021).

Critical COVID-19 patients may exhibit systemic hyperinflammation caused by macrophage activation syndrome or cytokine storms. The IL-6 profile analysis of the COVID-19 patients with severe symptoms showed that there were increased IL-6 levels among the nonsurvivors compared to the survivors. This increase was correlated with the occurrence of systemic inflammatory response syndrome and multi-organ failure (Donoso et al. 2021).

IL-6 can act on all cell types by binding to the transmembrane or soluble IL-6 receptor (IL-6R). It can also form a complex with gp130 and activate the effector Janus kinase (JAK). Activation of several of these pathways will produce various biological effects. Some of the effects are the maturation of naive T cells into effector T cells, the expression of vascular endothelial growth factor (VEGF) in endothelial cells, and increased vascular permeability. Decreased myocardial contractility may also occur, which contributes to organ damage

and the risk of death (Mehta et al. 2020, Nugroho et al. 2021).

Comorbidity is a factor that influences cytokine levels. A study found that TNF-α and IL-6 increased significantly in patients with chronic kidney disease, diabetes, hypertension, and a history of atrial fibrillation (Luporini et al. 2021). Another study conducted in New York, USA, found that comorbidity was the main cause of elevated IL-6 levels in patients aged 65 and above (Del et al. 2020). These findings may raise awareness of the important role of comorbidities in COVID-19 risk stratification.

IL-6 is a soluble mediator that has pleiotropic effects on inflammation, immunological response, and hematopoiesis. Physical exercise may cause the production of IL-6, which causes inflammation. IL-6 is considered one of the major pro-inflammatory interleukins and is involved in many immunological processes, including the induction of the acute phase response (Tanaka et al. 2014, Purwani et al. 2021).

Several studies have reported the association of high IL-6 levels with COVID-19 severity and poor outcomes. A study that examined serum IL-6 levels several times during treatment in the ICU reported higher IL-6 levels in nonsurvivor patients compared to survivors (Gorham et al. 2020). In this study, there was a significant difference in IL-6 levels on day 3 between the survivor and nonsurvivor groups. A study on 37 critical COVID-19 patients in Turkey supports these findings. The study reported that on day 3 of treatment, there was a significant decrease in IL-6 levels in the survivor group. In contrast, the nonsurvivors showed high and stable serum IL-6 levels. These dynamic changes, especially the decrease in serum IL-6 levels among the survivors, showed the role of cytokines in the critical prognosis of COVID-19 with a prospective follow-up (Ozger et al. 2021).

Fluctuations in IL-6 levels have a fairly short duration. A study stated that the peak of IL-6 levels in the survivor group was around 7–10 days from the onset of disease symptoms. Within 10 days of treatment, IL-6 levels return to normal (Santa et al. 2021). In this study, IL-6 levels reached a peak on day 3. This difference could be due to the timing of measuring IL-6 levels, which generally took place only when the patient was admitted to the hospital. Due to limited resources and the fact that IL-6 measurement is not routinely carried out, IL-6 measurement at first admission in severe or critical COVID-19 patients is very important to predict the possibility of complications and severity (Liu et al. 2020).

Strength and limitations

This study can contribute to COVID-19 pandemic management and control through patient stratification, laboratory tests, and IL-6 level analysis to evaluate patient severity and outcomes. The limitation of this study was the lack of time parameters for sampling because changes in inflammatory cytokine levels (e.g., IL-6) during infectious disease occurrence were quite dynamic. Another limitation of this study was the small sample size, consisting of only the patients who received lopinavir-ritonavir. Future research can be conducted on a larger scale for all COVID-19 patients.

CONCLUSION

Patients with critical COVID-19 and comorbidities, poor laboratory profiles, and high IL-6 levels are associated with a poor prognosis. Therefore, risk stratification, laboratory profiles, and IL-6 levels are important to assess the severity and outcomes of the patients.

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Conflict of interest

None.

Ethical consideration

This study was conducted with the approval from Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, with a reference number 149/113/ Komitlitkes/VII/2021 dated 15/7/2021.

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None.

Author contribution

EAT contributed to the conceptualization, study design, data curation, formal analysis, data interpretation, methodology, supervision, manuscript writing, and content revision. NMR contributed to the conceptualization, study design, data interpretation, methodology, investigation, manuscript writing, and content revision. N contributed to the conceptualization, study design, formal analysis, data interpretation, methodology,

investigation, project administration, writing, and content revision. FT contributed to the conceptualization, analysis, data interpretation, investigation, methodology, project administration, manuscript writing, and content revision.

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Original Research Report

SELECTED VEGETABLES AND SPICES IMPROVE DNA QUALITY AND HISTOPATHOLOGICAL ABNORMALITIES IN ROOF RATS (*Rattus rattus*) EXPOSED TO CEMENT DUST

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ABSTRACT

Conventional pollution control strategies in the cement industry have proven ineffective. As a result, effective and targeted complementary interventions are necessary. This study used roof rats (*Rattus rattus*) inhabiting the premises of a cement plant in Sagamu, Ogun State, Nigeria, to evaluate the ameliorative effects of moringa (*Moringa oleifera*), roselle (*Hibiscus sabdariffa*), fluted pumpkin (*Telfairia occidentalis*), and ginger (*Zingiber officinale*) on cement dust exposure. A total of 42 rats were divided into seven groups, with each group consisting of six rats. Group 1 served as the negative control group and was not exposed to any substances, while group 2 served as the positive control group and received standard feed throughout the experiment. Meanwhile, groups 3, 4, 5, 6, and 7 served as the experimental group. Rats in these groups were fed with 400 mg/kg of ethanolic extracts of *Z. officinale*, *M. oleifera*, *T. occidentalis*, *H. sabdariffa*, and a mixture of the four extracts with a composition of 1:1:1:1, respectively, for 90 days. The plasma DNA concentrations, DNA purity, and lungs of the rats were examined before and after the experiment. Prior to the experiment, the exposed rats had higher plasma DNA concentrations and lower DNA purity, as well as severe fibrosis and congested alveoli in their lungs, compared to the unexposed rats. At the end of the experiment, the experimental groups showed a significant increase in DNA purity ($p \leq 0.05$) and a decline in plasma DNA concentrations compared to the positive control group. In addition, the experimental groups showed fewer histopathological abnormalities than the positive control group. The mixture of the extracts yielded the most favorable results, followed by the extracts of *Z. officinale*, *M. oleifera*, *T. occidentalis*, and *H. sabdariffa*, respectively. These findings suggested that the selected vegetables and spices have the properties to ameliorate the effects of cement dust exposure. Therefore, individuals residing in close proximity to cement plants are encouraged to consume these vegetables and spices.

Keywords: Cement dust, congested alveoli, DNA purity, pollution, *Zingiber officinale*

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Highlights:
1. This study identified feasible and affordable alternatives to ineffective conventional cement production pollution control strategies.
2. *M. oleifera*, *H. sabdariffa*, *T. occidentalis*, and *Z. officinale* are effective as personal interventions to ameliorate the effects of cement pollution.

INTRODUCTION

Humans can survive for some time without water and food, but only for a very limited time without air. Air is so essential to life that the average adult breathes in and out over 7 liters of air per minute while at rest, or approximately 11,000 liters of air per day (Discovery Health 2022). Unfortunately, natural and anthropogenic phenomena are increasingly polluting the air, increasing the risk of several diseases. Breathing polluted air can cause illnesses such as eye and nose burns, throat irritation,

and difficulty breathing (National Institute of Environmental Health Sciences 2022). Particulate matter in the air can predispose humans to cardiovascular and respiratory diseases. It poses a greater risk, particularly for asthmatic individuals (Manisalidis et al. 2020, World Health Organization 2022). Heavy metals and volatile compounds such as benzene and vinyl chloride can initiate carcinogenesis, reproductive anomalies, lung injuries, and brain and nerve damage. In extreme situations, these effects can even be permanent and cause death (Lelieveld et al. 2020, Li et al. 2021). Pollutants can reach the upper atmosphere, causing the ozone layer to deplete. The gradual depletion of the ozone layer harms the environment and increases the risk of skin cancer and cataracts (United States Environmental Protection Agency 2022a).

Nearly all industries pollute the air, and the cement industry is one of the top polluters. According to the United States Environmental Protection Agency (2022b), the cement industry ranked third on the most polluting industry list. The reason for this was that the cement industry emits over 500,000 tons of greenhouse gases annually. Moreover, particulate matter, heavy metals, and dioxin may be present in cement dust released by kilns powered by the burning of hazardous waste. Cement dust contains various contaminants that may be harmful if exposed to humans and animals in significant amounts. The contaminants typically include silicon oxide, aluminum trioxide, calcium oxide, ferric oxide, sand, and magnesium oxide (Yahaya 2014, Rahmani et al. 2018). Most of these substances can be hazardous to living organisms when they exceed regulatory limits. These substances in particular have been linked to cancer, organ injuries, skin and eye deformities, blood disorders, genetic problems, and respiratory disorders (Occupational Safety & Health Administration 2016, Ahmad et al. 2021). These and other health problems caused by cement dust pollution must be prevented or ameliorated, especially in Nigeria and other countries where the cement industry is booming because of growing demand.

In the cement industry, traditional pollution prevention and control measures include the use of dust filters and protective gadgets, as well as tree planting around cement plants. However, not so much success has been recorded from these strategies due to weak environmental protection laws, technical challenges, a lack of funds, and insincerity, among others (Yahaya et al. 2022). Therefore, a complementary, cheap, and simple strategy is needed to prevent or ameliorate the health consequences of cement dust pollution. Plant medicine is an area that has not been completely utilized in the management of pollutant exposure effects. This study assessed the effects of moringa (*Moringa oleifera*), roselle (*Hibiscus sabdariffa*), fluted pumpkin (*Telfairia occidentalis*), and ginger (*Zingiber officinale*) on the DNA concentration and purity, as well as the lungs of roof rats (*Rattus rattus*) collected on the premises of a cement plant in Sagamu, Ogun State, Nigeria. People living in Nigeria frequently consume these plants as both vegetables and spices. These plants contain large amounts of antioxidants and phytonutrients, which are helpful in maintaining a healthy lifestyle. This study aimed to assess the use of nutrients in these plants to tackle health hazards caused by cement dust.

MATERIALS AND METHODS

This study utilized 42 roof rats (*Rattus rattus*) with a mean weight of 200.45±4.30 g that were captured on the premises of a cement factory in Sagamu, Ogun State, Nigeria. The rats were kept in metal cages for seven days to acclimate to the ambient environment prior to commencing the study, according to the method used in a study by Yahaya et al. (2022). The rats had free access to standard animal feed and water. The plant samples were sourced from a farm in Lagos, Nigeria, and identified in the herbarium section of the Department of Botany, University of Lagos, Nigeria. The authenticated samples were retained in the herbarium under the code numbers LUH 4558, LUH 4394, LUH 4395, and LUH 4396 for *M. oleifera*, *H. sabdariffa*, *T. occidentalis*, and *Z. officinale*, respectively.

The preparation to extract the bioactive components from the plants were carried out by following the guidelines in a study by Yahaya (2014). Powder (50 g) from each plant and the mixture were poured into 500 ml of 95% cold ethanol for 3 days (72 hours). The extracts obtained were filtered with muslin cloth and then dried to a constant weight at a 40±2°C temperature. The dry extract of each plant was dissolved in water and used for the experiment. The 'classical LD50' protocols as demonstrated in Anyebe et al. (2021) were followed to evaluate the acute toxicity of the plant extracts. In their study, 36 mixed-sex albino rats weighing from 205 to 210 g were utilized and divided into 6 groups of 6 rats each. After 12 hours of fasting, the rats in the treatment groups received 200, 400, 500, 1000, 3000, and 5000 mg/kg oral doses of the crude extracts, while the control groups were only given distilled water. The rats in each group were monitored for signs of toxicity for 24 hours.

The rats' DNA purity and plasma concentrations, as well as the lungs, were examined before administering treatments in this experiment (Suguna et al. 2014). The 42 rats were thereafter divided into

7 groups, each containing 6 rats. The negative control group (group 1) consisted of unexposed rats obtained from an area free from cement dust. The positive control group (group 2) consisted of rats exposed to cement dust but did not receive any treatment. Rats in groups 1 and 2, respectively, were given standard feed and water only. Rats in groups 3, 4, 5, 6, and 7 were fed with 400 mg/kg extracts of *M. oleifera*, *H. sabdariffa*, *T. occidentalis*, *Z. officinale*, and the mixture (1:1:1:1) of the four extracts for 90 days, respectively. The rats' DNA purity and plasma concentrations, as well as the histopathology of the lungs, were examined at the end of this study (Griffiths & Chacon-Cortes 2014).

Fresh blood pellet samples from the rats were collected using small vials (sample bottles) to determine the DNA purity. The blood samples were temporarily refrigerated, centrifuged for ten minutes at 5,000 rpm, washed twice, and then centrifuged again in 1,000 µl of phosphate-buffered saline (PBS). Afterwards, the blood pellets were transferred into an Eppendorf tube using a side-mouth disposable pipette and centrifuged at 10,000 rpm for five minutes (Suguna et al. 2014). The supernatants were retained and stored in a refrigerator at -40°C until required.

DNA was extracted from the blood pellets for isolation following the procedures described by Griffiths & Chacon-Cortes (2014). After transferring 200 µl of blood pellets into each of the labeled (001-024) Eppendorf tubes, 200 µl of 20% sodium dodecyl sulfate (SDS) was added to each tube and properly shaken. The homogenized mixture was placed in a heated bath (65°C) and intermittently shaken for 30 minutes before being removed and left to cool. Thereafter, 500 µl of ice-cold 5 M potassium acetate was transferred into each tube and shaken several times for proper mixing. After incubating the mixture on ice for 30 minutes, it was processed in a refrigerated centrifuge at 12,000 rpm for 10 minutes. The mixture in the tubes separated into two layers: the liquid portion at the top containing DNA (supernatants) and the solid portion at the bottom (residue) (Griffiths & Chacon-Cortes 2014).

The supernatants from each sample were properly transferred into two tubes per sample. The tubes were then filled with 1,000 µl of ice-cold isopropanol and delicately shaken until DNA strands were visible. The tubes were left to stand for a while before being incubated for 24 hours at -20°C in a freezer to ensure complete precipitation, as characterized by the appearance of a white mass in the liquid mixture (Griffiths & Chacon-Cortes 2014). The supernatants were decanted, leaving the strands in the tubes. The tubes were placed face down on paper towels for an hour in order to remove the final drops of isopropanol. The DNA strands were then washed by mixing them with 70% ethanol and centrifuging them at 12,000 rpm for 10 minutes at 4°C. The ethanol was discarded, and the tubes were inverted for ten minutes to remove the leftover ethanol. The tubes were then placed flat on paper towels for the pellets to dry completely over the course of four hours. The DNA was dissolved in 100 µL of double-distilled water and refrigerated before further analysis (Griffiths & Chacon-Cortes 2014).

Spectrophotometry was conducted to measure the rats' DNA quality and quantity using the BioPhotometer Plus (Eppendorf, A.G., Hamburg, Germany). The quantity was the concentration of the DNA in nanograms per microliter (ng/µl), while the quality (purity) was the absorbance of ultraviolet light through the cuvette measured in Armstrong (Å). The cuvette was an accessory that held the diluents (sterilized filtered water) and DNA material (Potter & Heller 2018). The equipment was activated and scrolled to the double-stranded DNA setting, which was calibrated to the quantity of materials being tested. The diluents used to elute or dilute the DNA were also used to reduce its concentration in order to obey the Beer-Lambert Law (concentration of double-stranded DNA=A260 × 50 µg/µl) (Grasse et al. 2016). The dilution ratio for the DNA and diluents was 5:95 µL, with a total volume of 100 µL. The cuvette was rinsed with the same diluents and standardized prior to taking the reading for spectrophotometer analysis. The DNA was properly introduced and homogenized before pressing the sample button and obtaining a reading. On the liquid crystal display (LCD), the results for concentration (ng µL-1), simultaneous absorbance at three different wavelengths (230A, 260A, and 280A), and a ratio of 260A/280A were shown to assess the purity of the sample.

The guidelines outlined in Anyebe et al. (2021) were followed to prepare the rats' lungs for histopathological examinations. After the tissues were excised, the routine hematoxylin and eosin staining technique was used for preparation. Following the staining, observation of the tissue was conducted under a light microscope (40X magnification), with emphasis on the cytology, architecture, and morphology of the tissues. In the data analysis, IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) was used to compute the values obtained as mean±standard deviation. Comparison of the differences between the treatment and control groups was done using the F-test (ANOVA), and $p \leq 0.05$ was considered a significant difference.

RESULTS

The acute toxicity tests revealed that the extracts were non-toxic to the rats at all the doses administered. A dose of 400 mg/kg of body weight seemed to produce the best results. Therefore, the dose was chosen for subsequent analyses. General observations for 24 hours after extract administration presented no mortality. The administration of *Z. officinale*, *M. oleifera*, and *T. occidentalis* extracts, as well as the mixture of plant extracts, exhibited no signs of distress or excitement among the rats. The rats that *received H. sabdariffa*, on the other hand, licked the cannula aggressively, which was an indication that they relished the extract.

Regarding the effects of plant extract administration on the roof rats, Table 1 shows the DNA concentrations and absorbance (purity) with optical densities at 260/280. The DNA purity of the treated rats improved significantly ($p \leq 0.05$) compared to those that only received water. The absorbance was measured to be 1.65 in the negative control group (group 1), 1.39 in the positive control group (group 2), 1.51 in the group receiving *M. oleifera* extract (group 3), 1.43 in the group receiving *H. sabdariffa* extract (group 4), 1.52 in the group receiving *T. occidentalis* extract (group 5), 1.63 in the group receiving *Z. officinale* extract (group 6), and 1.60 in the group receiving a mixture of the plant extracts (group 7).

Table 1. DNA purity of the rats fed with plant extracts for 90 days.

Extract	DNA conc. (ng/µl)	260	Absorbance (A) 280	260/280
Group 1	2.11±0.91	0.028±0.001	0.018±0.006	1.65±0.08
Group 2	6.21±1.82	0.031±0.002	0.023±0.001	1.39±0.61
Group 3 (*H. sabdariffa*)	4.21±1.25	0.022±0.012	0.016±0.001	1.43±0.01*
Group 4 (*M. oleifera*)	3.32±0.73	0.018±0.002	0.012±0.001	1.51±0.11*
Group 5 (*Z. officinale*)	2.31±0.22	0.029±0.002	0.018±0.001	1.63±0.13*
Group 6 (*T. occidentalis*)	3.42±0.93	0.031±0.014	0.021±0.016	1.52±0.11*
Group 7 (Mixture of extracts)	2.35±0.53	0.048±0.001	0.030±0.002	1.60±0.16*
Rat baseline	7.56±2.51	0.052±0.007	0.039±0.003	1.30±0.02

Values were expressed as mean±SD; group 1: non-exposed rats (negative controls) obtained from a cement-dust-free area; group 2: exposed rats treated with only water; rat baseline: DNA quality of the exposed rats a day before treatment; if absorbance (A) at 260/280 is 1.5–2.0, it is normal; if absorbance <1.5 and >2.0, it is abnormal; values with asterisks (*) are significantly different from the negative control group (group 2), with $p \leq 0.05$ (ANOVA).

After administering the plant extracts to the rats, the lung tissue was examined to determine the extracts' effects. Figure 1 shows normal alveoli that were found in the lungs of the rats that lived in an area free from cement dust. Figure 2 reveals the lungs of the exposed rats prior to extract administration, showing severe inflammation, vascular congestion, a congested alveolus, and severe fibrosis. Figure 4 demonstrates the improvement of the rats' lungs following the administration of the extracts. After the administration of *H. sabdariffa* extract, the lung tissue of the rats showed congested alveoli and moderate inflammation. There was moderate inflammation in the lung tissues of the rats after *M. oleifera* extract was administered. Mild inflammation was also present in the lung tissue of the rats that received *Z. officinale* extract. Mild vascular congestion was observed in the lungs of the rats treated with *T. occidentalis* extract. The lung tissue of the rats that received a mixture of the extracts showed mild inflammation. However, the exposed rats that received only water still revealed severe fibrosis (Figure 3).

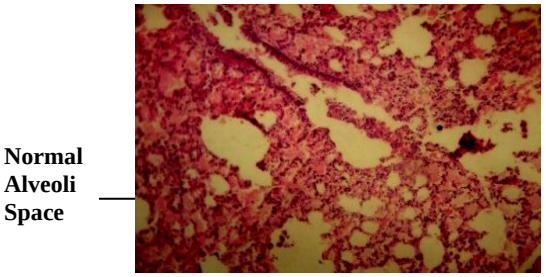

Figure 1. Lung histopathology of the rats obtained from an area without cement dust (400X magnification).

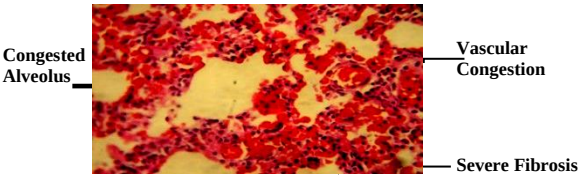

Figure 2. Lung histopathology of the exposed rats prior to extract administration (400X magnification).

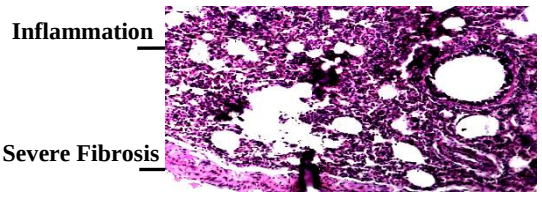

Figure 3. Lung histopathology of the control rats administered with distilled water (400X magnification).

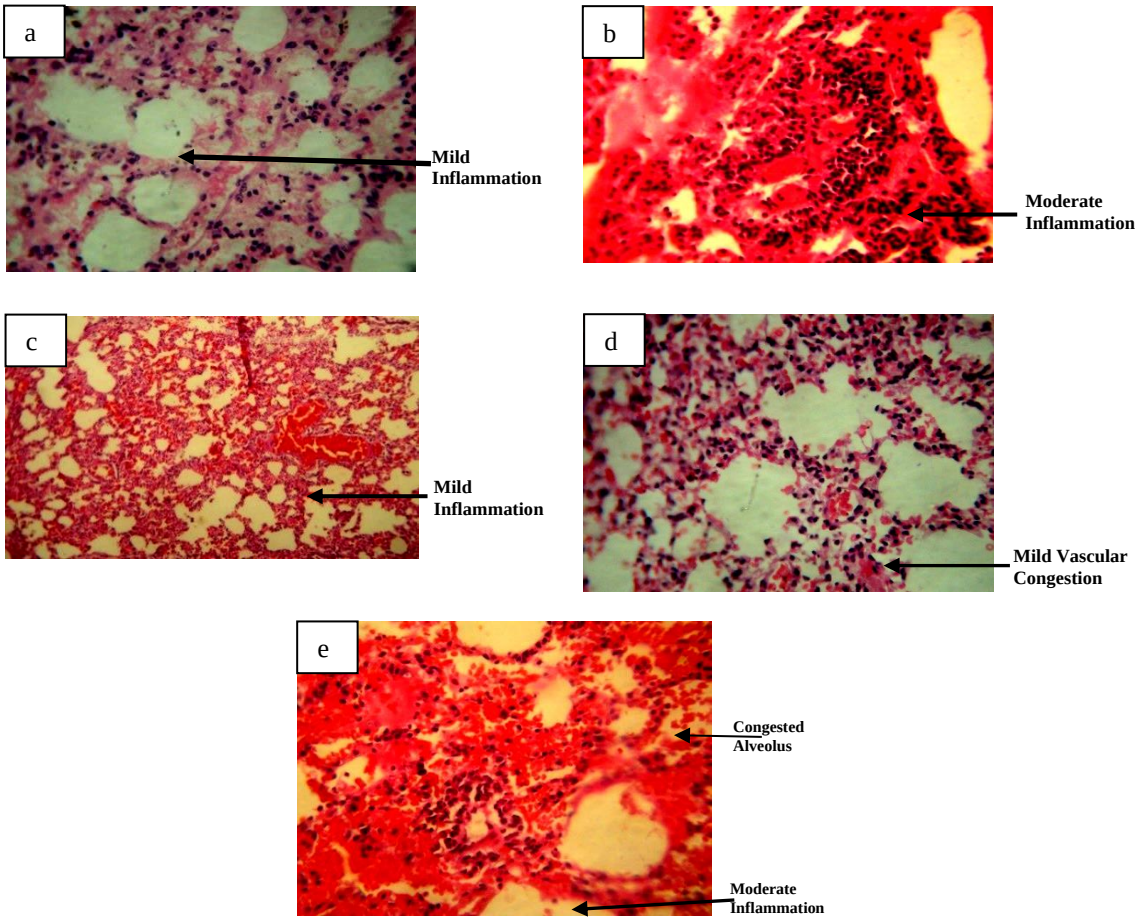

Figure 4. Lung histopathology of the rats administered with *Hibiscus sabdariffa* extract (4a), *Moringa oleifera* extract (4b), *Zingiber officinale* extract (4c), *Telfairia occidentalis* extract (4d), mixture of plant extracts (4e) at 400X magnification.

DISCUSSION

This current study evaluated the ameliorative effects of *M. oleifera*, *H. sabdariffa*, *T. occidentalis*, and *Z. officinalis* extracts on roof rats residing in the vicinity of a cement plant. This study was conceptualized to find a nutrient-based and individualistic approach to the health hazards associated with cement dust exposure. Rats exposed to cement dust had high plasma DNA concentrations. This is an indication of oxidative stress-induced DNA damage, which is a biomarker for a number of diseases, including cancer, trauma, myocardial infarction, stroke, and sepsis, among others (Pizzino et al. 2014, Graille et al. 2020). Plasma DNA are DNA fragments that are detectable in extracellular fluid and are present at very low levels in healthy individuals. DNA fragments enter the circulation following cell death, either through cell necrosis or apoptosis (Pizzino et al. 2014, 2017).

The low DNA purity of the exposed roof rats indicated that their DNA had been contaminated and that their health had consequently been compromised by the poisonous components of cement dust. A previous study reported that the bloodstreams of rats exposed to cement dust contained potentially toxic elements, mainly heavy metals, aluminum, and calcium (Yahaya 2014). These elements have been linked to several diseases. In rats exposed to chromium and lead in mining areas, overexpression of certain mRNAs was observed in the blood of the animals (Nakayama et al. 2013). An observation of the germinal cells of rats exposed to lead and cadmium revealed DNA damage in the spermatocytes. Exposure to aluminium oxide and silicon oxide nanoparticles has been demonstrated *in vitro* to cause DNA damage (Hashimoto & Imazato 2015, Adamkovicova et al. 2016). The results of the current study were in line with earlier studies that investigated cement dust exposure (Krishna et al. 2020, Akiibinu et al. 2016). Detected biomarkers in workers exposed to cement dust indicated DNA damage and increased cell death. Workers exposed to cement dust experienced an occupational hazard that led to DNA damage characterized by raised plasma 8-OHdG levels.

The improved DNA purity and reduced plasma concentrations following treatment using plant extracts could be attributed to the phytochemicals and phytonutrients in the plants. A phytoconstituent analysis of the examined vegetables revealed the presence of numerous vitamins and minerals (Yahaya et al. 2017). Health-boosting phytochemicals (such as alkaloids, flavonoids, tannins, phlobatanins, glycosides, and saponins) and reducing sugars were also detected. It has been demonstrated that plants containing the mentioned phytonutrients and phytochemicals, such as the vegetables and spices evaluated in this study, enhance health and prevent health risks. In an experiment, *H. sabdariffa* showed genoprotective effects against H2O2-induced DNA damage. Aqueous extracts of *M. oleifera* leaves, seeds, and fruits have also been demonstrated to inhibit oxidative DNA damage (Abdul et al. 2014, Karim et al. 2016). Other studies reported that *Z. officinale* extract reduced plasma DNA concentrations and damage in rats with heavy metal-induced oxidative damage. Oral administration of *T. occidentalis* seed oil has been shown to ameliorate DNA damage caused by azathioprine-induced oxidative stress (Shaban & Sahu 2017, Gabr et al. 2019, Okesola et al. 2020).

The histopathological damage observed in the exposed rats' lungs demonstrated the deleterious effects of cement dust since the lungs are the first to contact with an inhaled substance. Earlier studies revealed lung damage in humans and rats exposed to dust from cement plants (Rahmani et al. 2018, Owonikoko et al. 2021). Furthermore, Richard et al. (2016) reported reduced lung function in workers exposed to cement dust. As already mentioned earlier, the heavy metals in cement dust could be responsible for the histopathological damage. Studies have discovered that being exposed to silica can potentially cause damage to the lungs (Occupational Safety & Health Administration 2016, Almansour et al. 2018). Welders have been reported to suffer from interstitial lung disease, upper lobe fibrosis, and peripheral emphysema as they are occupationally exposed to aluminum dust. There was evidence of lung inflammation, injury, and proliferative responses in rats that were repeatedly exposed to hexavalent chromium (Feary et al. 2020, Zhang et al. 2023). Acute and chronic lead exposure have been demonstrated to cause lung damage. Calcium is a mineral that is essential for both humans and animals. However, a biologically foreign form of calcium, such as that found in cement dust, can cause organ damage when deposited in the wrong organs (Baccarelli et al. 2014, Offor et al. 2017, Attafi et al. 2022).

The improvement observed in the rats' lungs following the extract administration can be attributed to the phytoconstituents mentioned previously. Protective effects of *M. oleifera* on lung functions, hematological damage, and lipid damage induced by heavy metals have been demonstrated using experimental rats. Ameliorative effects of *H. sabdariffa* on pulmonary fibrosis and chronic inflammatory interstitial lung disease have also been studied using rats exposed to a toxic chemical (Hemmeti et al. 2016, Ajibade et al. 2021). It has been demonstrated that pre-treatment using *Z. officinale* extract improves lung function and normalizes lung injuries in alcoholic humans and rats exposed to polychlorinated biphenyls (Alireza et al. 2017, Ahd et al. 2019). The administration of *T. occidentalis* extract to animals resulted in the repair of lung tissue damage. In addition, parameters of liver function revealed beneficial effects of the extract (Chukwuemeka et al. 2020, Saadat et al. 2022). This study complements the previous studies by demonstrating that the mixture of the four plant extracts in equal ratios worked better than each plant extract. This suggests that the phytoconstituents in the plants work synergistically to enhance the efficacy of the plant extracts.

Strength and limitations

The study reflects the practical implications of exposure to cement dust in a typical cement production environment by employing local rats that were cost-efficient on the premises. This implication was strongly supported at the molecular level, as the DNA purity check indicated the extent of cement pollution for anyone living in the vicinity of the cement factory. However, the limitation of this study was that only a representative sample was collected, which might not accurately reflect the entire population around the cement site.

CONCLUSION

The extracts of *M. oleifera*, *H. sabdariffa*, *T. occidentalis*, and *Z. officinale* can enhance DNA purity and alleviate histopathological damage in the lungs of rats exposed to dust from a cement plant. The therapeutic efficacy of these vegetables and spices may be enhanced by combining them in a mixed formula. Individuals working or residing in close proximity to cement plants are advised to incorporate these vegetables and spices into their diet.

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Conflict of interest

None.

Ethical consideration

This study was conducted in accordance with the ethical standards of European and German animal welfare legislation, the Declaration of Helsinki, and the National Institutes of Health guidelines for the care and use of animals in research. The local ethics committee of the Federal University, Birnin Kebbi, Nigeria, approved all protocols used in this study (Regulation CEE 86/609 dated 3/12/2022).

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None.

Author contribution

TOY conceptualized, designed, and drafted the manuscript. TFS analyzed and interpreted the data as well as performed a critical revision of the manuscript for important intellectual content. CO granted the manuscript final approval. AI provided the study materials. MNM collected and compiled the data. SSR conducted the statistical analysis. SA provided administrative, technical, and logistic support.

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Original Research Report

TREATMENT OF ACUTE DIARRHEA IN CHILDREN AGED 1–5 YEARS PROVIDED BY DOCTORS IN SURABAYA

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ABSTRACT

Diarrhea is the second leading cause of death among children under five, following pneumonia. Insufficient knowledge of diarrhea and dehydration management may contribute to this high mortality rate. Implementing prompt and effective management and prevention strategies has the potential to decrease morbidity and mortality associated with diarrhea. Therefore, this cross-sectional study aimed to investigate the treatment methods used by doctors for children aged 1–5 suffering from acute diarrhea in Surabaya, Indonesia. Data were collected from doctors in Surabaya using an online form containing case-based questions. Microsoft Excel for Mac version 16.17 (Microsoft Inc., Redmont, WA, USA) was used to process the binary data by calculating frequencies and percentages with a point estimate of 95% confidence interval (CI). A descriptive method was used in the data analysis, and the findings were presented in tables. From a total of 51 respondents who participated in this study, 18 (35.29%) only administered oral rehydration therapy. In addition, 49 respondents (96.08%) prescribed zinc supplementation for ten days, while 10 respondents (19.61%) opted for antibiotics. Interestingly, 49 respondents (96.08%) recommended both breast milk and food for the patients, and nearly all respondents provided guidance to the mother or caregiver of the patient. The findings of this study suggested that the respondents primarily used rehydration therapy to treat children suffering from acute diarrhea with moderate dehydration. However, some respondents still relied on antibiotics despite the available recommendations to use oral rehydration therapy. Nearly all respondents agreed on zinc supplementation, breast milk and food provision, and advice for the mother or caregiver of the patient. While certain aspects of the disease management for diarrhea align with the recommended guidelines, there is room for improvement in promoting the exclusive use of oral rehydration therapy and reducing unnecessary antibiotic prescriptions.

Keywords: Diarrhea; children; guidelines; case-based questionnaire

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Highlights:
1. The focus of this study was on the treatment methods for acute diarrhea, which have not been extensively investigated in the region studied.
2. This study can provide an overview of doctors' adherence to the available guidelines for the treatment of acute diarrhea in children aged 1–5 years in Surabaya, Indonesia.

INTRODUCTION

Diarrhea is one of the leading health problems among pediatric patients worldwide. According to data from the World Health Organization (WHO) in 2017, around 525,000 children died due to diarrhea. This placed diarrhea as the second leading cause of death among children under five, after pneumonia (World Health Organization 2017). Children in developing countries continue to face significant health and survival risks. One of the challenges threatening the health and survival of children is

acute diarrhea, which remains a primary cause of morbidity and mortality (Akuffo et al. 2017). Children under the age of 5 have a higher incidence of acute diarrhea due to their susceptibility to viral infections. Data from the Indonesian Ministry of Health in 2018 revealed that the highest prevalence of diarrhea in Indonesia occurred in the age group of 1–4 years (Minister of Health of the Republic of Indonesia 2019).

The WHO recommends rehydration using low-osmolarity oral rehydration solutions and zinc administration as the first treatments for diarrhea. The recommendations are based on the low cost, safety, and convenience of the treatments for treating diarrhea (World Health Organization 2017). The European guidelines state that oral rehydration with a hypotonic solution is the primary treatment and should be administered as soon as possible. The guidelines also recommend that breastfeeding should not be stopped and that regular feeding without dietary changes should continue (Guarino et al. 2018).

In many countries, oral rehydration solution is the initial remedy for diarrhea. In the United States of America (USA), oral rehydration solution is also recommended as a first-line therapy for diarrhea with mild to moderate dehydration from any cause. (Shane et al. 2017). In India and the Maldives, oral rehydration solution is the first-line therapy for all children with acute diarrhea. Additionally, zinc supplementation is given for 10–14 days to relieve the symptoms and help with the patient's recovery (Bishwakarma 2022, Cherukuri et al. 2022).

The guidelines for acute diarrhea in Indonesia are similar to the aforementioned guidelines. The Indonesian Ministry of Health has developed five steps for managing diarrhea in children (*Lima Langkah Tuntaskan Diare*, shortened as LINTAS DIARE), which consist of: (1) administering oral rehydration therapy; (2) providing zinc tablets for ten consecutive days; (3) continuing to provide breast milk and food; (4) selectively using antibiotics; and (5) advising mothers and families (Minister of Health of the Republic of Indonesia 2012).

However, despite the availability of guidelines, doctors' compliance with those instructions is still lacking in many regions. A study conducted at a hospital in Nairobi, Kenya, reported that compliance with clinical guidelines is still relatively low (Shitemi 2018). Another study conducted at a tertiary hospital in western India revealed numerous deviations from the WHO recommendations, including the administration of probiotics, anti-diarrheal medications, antibiotics, and intravenous rehydration. In eastern India, doctors' compliance with diarrhea treatment guidelines for infants under the age of 5 was also low. These deviations occurred frequently, although the WHO recommends the administration of oral rehydration solution and zinc (De et al. 2016, Behera et al. 2021).

Effective management and prevention programs can reduce mortality and morbidity rates associated with diarrhea. Doctors' compliance with the guidelines for diarrhea treatment is crucial for the realization of this idea (Indriyani & Putra 2020). Unfortunately, there is currently no data available on the compliance rate with the LINTAS DIARE guidelines in Indonesia. This research aimed to describe the management of acute diarrhea in children aged 1–5 years in Surabaya, Indonesia, according to the LINTAS DIARE guidelines (Minister of Health of the Republic of Indonesia 2012). The findings of this research would be important in urging further evaluation and control. Aside from that, this study had to be conducted to improve clinical services and research functions on related topics, so that the obtained data could serve as a basis for future research in other regions of Indonesia.

MATERIALS AND METHODS

This article presents a cross-sectional study conducted between September and November 2022 using online case-based questionnaires based on the study by Ke et al. (2012) and distributed to doctors in Surabaya, Indonesia. The research population comprised of 34 general practitioners and 17 pediatricians in Surabaya, Indonesia, who have ever treated acute diarrhea in children aged 1–5 years. The inclusion criteria were general practitioners and pediatricians who treated acute diarrhea in children aged 1–5 years with any degree of dehydration and were willing to complete the questionnaire. Participants with insufficient data who were unable to complete the questionnaire were excluded.

A questionnaire referred to the study by Ke et al. (2012) was created on Google Forms and distributed via WhatsApp to general practitioners and general pediatricians in Surabaya. The questionnaire consisted of two sections, with a total of eight demographic data questions and nine case-based questions. The first section consisted of questions regarding the respondents' willingness to complete the questionnaire, age, gender, level of qualification (general practitioners or general pediatricians), practice location (outpatient clinic, inpatient ward, or emergency unit), the average number of pediatric acute diarrhea patients per day in outpatient clinics and inpatient wards, and whether they routinely treat pediatric patients aged 1–5 years. In the second section of the questionnaire, the respondents were

given a hypothetical scenario, i.e., acute diarrhea with moderate dehydration, according to the guidelines from the (World Health Organization 2013).

The questions should be answered based on the respondents' experiences in handling such cases. One of the questions was regarding the therapy plan they typically provide for patients with moderate dehydration. The presented case involved a 3-year-old child who came to the hospital agitated and fussy. This patient had been experiencing diarrhea for five days with an axillary temperature of 36.5 °C. Furthermore, the patient felt thirsty and wanted to drink a lot. When the pinch test was performed, it showed that the patient had poor skin turgor as the skin returned to its position slowly. After reading the case, the respondents were asked to answer nine questions related to the treatment plan for the case. These questions could be answered with "yes", "no", or "do not know" (World Health Organization 2013). The questionnaire queries are presented within tables in this article.

Data obtained from respondents were processed using Microsoft Excel for Mac, version 16.17 (Microsoft Inc., Redmont, WA, USA). A statistical analysis was performed with a point estimate at 95% confidence interval (CI) to calculate the frequency and percentage of the binary data (Maskey et al. 2019). This data analysis utilized a descriptive method by classifying the data according to the research variables. The tables in this article present the data to demonstrate the comparison of the treatments administered by doctors to acute diarrhea patients with moderate dehydration.

RESULTS

There were 51 respondents, consisting of general practitioners and general pediatricians who provide treatment for acute diarrhea in children aged 1–5 years in Surabaya. Table 1 displays the general characteristics of the respondents. Most respondents, as many as 29 (57%), were younger than 40 years old. Women made up the largest proportion of the respondents, accounting for 35 (69%) participants. The level of qualification for most respondents was general practitioner, with 34 (67%) respondents. A total of 27 (52%) respondents worked in outpatient clinics. Of the respondents, 37 (73%) reported that they had been treating up to ten cases of acute diarrhea in children.

Table 2 shows the respondents' responses to the questions regarding acute diarrhea treatment. In answering the case-based questionnaire, only 18 (35.3%) respondents would provide oral rehydration therapy solely, while 14 (27.5%) would provide intravenous rehydration therapy, and 19 (37.2%) would provide both oral and intravenous rehydration therapy. In addition to oral rehydration, 49 (96.1%) respondents would provide zinc supplementation for 10 days. However, 10 (19.6%) respondents would still administer antibiotic treatment for acute diarrhea.

Almost all participants would advise continuing breast milk and food provision and not stopping them until diarrhea improved. They would also provide education to mothers and caregivers on how to administer oral rehydration and zinc supplementation, educate mothers and caregivers on breast milk or food provision, and explain the signs that a child should be taken to a medical professional immediately.

Table 1. General characteristics of the respondents.

Characteristics	Frequency (n)	Percentage (%)
Age		
<40 years	29	57
≥40 years	22	43
Sex		
Male	16	31
Female	35	69
Qualification level		
General practitioners	34	67
General pediatricians	17	33
Practice location		
Inpatient ward	12	24
Outpatient clinic	27	52
Emergency unit	12	24
Number of pediatric patients with acute diarrhea each day		
0–10 patients	37	73
11–20 patients	13	25
>20 patients	1	2

Although the majority of respondents would choose rehydration therapy and zinc administration to treat acute diarrhea, some would also prescribe antibiotics. As shown in Table 3, four respondents who would administer antibiotics chose cotrimoxazole for cases of acute diarrhea with mild to moderate dehydration. One respondent chose two antibiotics, i.e., cotrimoxazole and cefixime.

Table 2. Responses to the questionnaire regarding cases of acute diarrhea with moderate dehydration.

Questions	Yes (%)	No (%)	Do not know (%)
Oral rehydration therapy only	18 (35.3)	33 (64.7)	0 (0)
Intravenous rehydration therapy only	14 (27.5)	37 (72.5)	0 (0)
Both oral and intravenous rehydration therapy	19 (37.2)	32 (62.8)	0 (0)
Zinc administration for 10 days	49 (96.1)	2 (3.9)	0 (0)
Antibiotic administration	10 (19.6)	40 (78.4)	0 (0)
Continuation of breast milk and food provision	49 (96.1)	0 (0)	2 (3.9)
Advising not to stop breast milk and food provision until diarrhea improves	0 (0)	49 (96.1)	2 (3.9)
Educating the mother and caregiver about how to administer oral rehydration salt and zinc	51 (100)	0 (0)	0 (0)
Educating the mother and caregiver about breast milk or food provision	50 (98.0)	1 (2)	0 (0)
Explaining the signs that the child should be taken immediately to a health professional	51 (100)	0 (0)	0 (0)

Table 3. Types of antibiotics frequently prescribed by the respondents for cases of acute diarrhea with mild to moderate dehydration.

Antibiotics	Frequency (%)
Cotrimoxazole	4 (7.8)
Amoxicillin	2 (3.9)
Cefixime	2 (3.9)
Metronidazole	2 (3.9)
Nifuroxazide	1 (1.9)

DISCUSSION

In this study, many respondents still provided therapies other than oral rehydration therapy. The majority of the respondents combined oral rehydration therapy with intravenous rehydration therapy. The WHO stated that treating diarrhea aims to prevent dehydration, manage dehydration, prevent malnutrition, and reduce the severity, duration, and likelihood of future episodes (World Health Organization 2013). In the LINTAS DIARE guidelines, treatment plan B is given to patients with acute diarrhea and mild to moderate dehydration. However, fluid administration using oral rehydration therapy is prioritized (Minister of Health of the Republic of Indonesia 2012).

Many guidelines state that intravenous rehydration should only be administered in exceptional circumstances. Intravenous rehydration can be beneficial in cases of shock, severe dehydration, dehydration with an altered state of consciousness or severe acidosis, persistent vomiting, and severe abdominal distension and ileus (Minister of Health of the Republic of Indonesia 2012, World Health Organization 2013, Shane et al. 2017). The guidelines in Europe, America, India, and the Maldives recommended only oral rehydration therapy using low-osmolarity oral rehydration solutions for acute diarrhea with moderate dehydration. Although intravenous rehydration has been used for a long time, it is recommended only for cases of acute diarrhea (Guarino et al. 2014, Bishwakarma 2022, Cherukuri et al. 2022). When the patient is hydrated and conscious, intravenous rehydration should be stopped immediately and replaced with oral rehydration. Intravenous rehydration should be avoided in severely malnourished patients (Guarino et al. 2014, Brandt et al. 2015).

The findings of this study were comparable to those of a study by Adiba et al. (2022) at a tertiary hospital in Surabaya, which revealed that mild and moderate were the most prevalent degrees of dehydration. However, the most common method of rehydration used was intravenous rehydration. Intravenous fluids are so common in hospitals that it is easy to overlook the signs of patients who require them (Toaimah & Mohammad 2016, Gawronska et al. 2022). Many doctors prefer intravenous rehydration because oral rehydration therapy requires more time. In oral rehydration therapy, the patient requires an extended stay in the emergency unit, while the staff requires longer working time. The fact that some respondents in a study worked in the emergency unit and inpatient ward made them prefer intravenous rehydration in the emergency unit (Gawronska et al. 2022).

Intravenous fluids have the advantage of being able to be administered directly into the bloodstream. Therefore, time delays that can occur with oral rehydration procedures can be eliminated (Gawronska et al. 2022). Another reason that leads to the widespread use of intravenous rehydration is a fault in the initial assessment of the patient's dehydration degree. Determining the initial evaluation of dehydration degree is therefore critical

in deciding its therapy (Abdul-Mumin et al. 2019). The results of this study are identical to those of the study by Ozuah et al. (2002), in which many doctors did not solely use oral rehydration therapy for cases of moderate dehydration in acute diarrhea, with only 15.3% of participants using oral rehydration therapy. The study reported several reasons that drive this trend, including patient refusal to drink fluids, vomiting as the primary symptom, inability to urinate, presence of ketonuria, parental concerns about dehydration, crowded emergency units, and increased urine specific gravity.

This study showed that 49 respondents would administer zinc for 10 days, but there were still some who would not provide it. Previous studies researched the effect of zinc supplementation on diarrhea and found that it has a preventive and long-term impact. Taking 10–20 mg of zinc daily for 10–14 days can help reduce the severity of diarrhea and prevent future occurrences for up to 2–3 months after the supplementation period. This aligns with the recommendations on diarrhea treatment by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) that the administration of oral rehydration salts and zinc for 10–14 days, with 20 mg of zinc daily for children with acute diarrhea and 10 mg for infants under 6 months. Zinc can be given in the form of chewable zinc tablets or by dissolving it in one spoonful of boiled water or breast milk (Kassa et al. 2022).

Recent studies suggest that zinc supplementation should be given to malnourished or zinc-deficient children between the ages of 6 months and 5 years who live in developing countries or areas where the prevalence of mild to severe zinc deficiency is high (Shane et al. 2017, Guarino et al. 2018). However, zinc is ineffective in children under 6 months, regardless of their nutritional status. Although zinc supplementation does not decrease stool volume considerably, it was related to a significant reduction in diarrhea duration and the likelihood of diarrhea lasting more than seven days (Guarino et al. 2014). Zinc can increase the body's immune system, thus preventing the risk of recurrent diarrhea for 2–3 months after the child recovers from diarrhea. According to a report spanning 18 years by the WHO, the benefits of zinc as a diarrhea treatment include reducing diarrhea prevalence by 34%, reducing acute diarrhea duration by 20%, reducing persistent diarrhea duration by 24%, and reducing therapy failure or death due to persistent diarrhea by 42% (Minister of Health of the Republic of Indonesia 2012). Some respondents would not provide zinc due to their fear of possible side effects. These side effects were found to occur more often in children and infants who were given zinc compared to those who were not. The most common side effect of zinc administration is vomiting (Shane et al. 2017). The other adverse effects may include stomach trouble, heartburn, nausea, fever, sore throat, mouth sores, weakness, and fatigue. These adverse effects can be burdensome for children suffering from diarrhea as well as for their parents (Kassa et al. 2022).

In this study, 10 respondents would still prescribe antibiotics in the case-based questionnaire. Antibiotics should not be needed routinely for acute diarrhea, even if bacteria are suspected of causing the condition, because most cases of acute diarrhea are self-limiting within a few days. Inappropriate antibiotic use can prolong diarrhea duration due to intestinal microflora dysregulation (Minister of Health of the Republic of Indonesia 2012, Guarino et al. 2018). Children are susceptible to viral infections such as the common cold, influenza, ear infections, and diarrhea. The most common cause of diarrhea in children is infection by viruses, specifically rotavirus and norovirus. Therefore, antibiotic treatment is unnecessary for most diarrhea cases (Sudarmo et al. 2015, Athiyyah et al. 2019). This is more prevalent in countries that are still developing. The overuse of antibiotics to treat diarrhea is a leading cause of rising rates of antibiotic resistance in developing countries (Kassa et al. 2022).

Antibiotic therapy can be considered for children under 3 years old with underlying chronic conditions (such as sickle cell anemia or immunodeficiency). It can also be considered for children with specific pathogenic infections (such as dysentery or cholera) (Guarino et al. 2018). A study conducted at a tertiary hospital in Surabaya showed a higher use of antibiotics for patients with diarrhea than in other hospitals. This is due to the high prevalence of comorbid infections among the patients that necessitate antibiotic therapy (Jordan et al. 2020). However, the fact that some respondents would still prescribe antibiotics indicated a lack of confidence in oral rehydration therapy and proper training in diarrhea management (Behera et al. 2021). This study's results showed that cotrimoxazole was the most commonly prescribed antibiotic medication. Cotrimoxazole is a sulfa antibiotic that inhibits bacterial growth so that the body's immune system can strengthen (Church et al. 2015).

Strength and limitations

This study may offer a general description of acute diarrhea treatment in children and the adherence of doctors to the available guidelines. This subject has not received sufficient attention from researchers in the area. However, this study only covered doctors in Surabaya, Indonesia, so further studies can be conducted on a larger scale and using more diverse and numerous scenarios.

CONCLUSION

This study revealed that many doctors did not exclusively rely on oral rehydration therapy, and antibiotics were still being prescribed despite the absence of indications for their use. It is crucial to emphasize the importance of doctors to adhere more closely to the available guidelines regarding the administration of rehydration therapy for children suffering from acute diarrhea with moderate dehydration. However, nearly all doctors agreed on zinc supplementation, breast milk and food provision, and advice for the mother or caregiver of the patient.

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Conflict of interest

None.

Ethical consideration

This study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia (No. 130/EC/KEPK/FKUA/2022 dated 23/7/2023).

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None.

Author contribution

AFA, IRI, and NF conceptualized and designed this study. IRI collected, analyzed, and interpreted the data, as well as drafted the manuscript. AFA and NF procured the statistical expertise, critically revised the manuscript for important intellectual content, and gave the final approval. AFA also interpreted the data, supplied the research materials and patients, and provided funding for this study. NF provided administrative, technical, and logistic support.

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Original Research Report

STRESS LEVELS AND SEBORRHEIC DERMATITIS IN THE CLASS OF 2020 MEDICAL STUDENTS AT A UNIVERSITY IN INDONESIA

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ABSTRACT

Seborrheic dermatitis is a common skin disease in the form of superficial inflammation with a papulosquamous morphology that often occurs in sebaceous gland-rich areas. Emotional stress is one of the contributing factors to the susceptibility of individuals to seborrheic dermatitis, while lack of sleep is associated with its exacerbation. This study aims to investigate the relationship between stress levels and seborrheic dermatitis cases among the class of 2020 students at the Faculty of Medicine, Universitas Tarumanagara, Jakarta, Indonesia. This study used an analytical-observational approach with a cross-sectional design. The sampling technique used was simple random sampling. The samples were from 114 medical students from the class of 2020 at Universitas Tarumanagara. The data were collected using an online Google Forms questionnaire and a dermatological assessment. The data were analyzed through statistical software, IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, N.Y., USA), using the Chi-square hypothesis test. The results showed a significant relationship between sex and seborrheic dermatitis cases (p=0.000). However, there was no significant relationship between stress levels and seborrheic dermatitis cases among the respondents (p=0.591). In conclusion, factors other than stress levels might contribute to the development or exacerbation of seborrheic dermatitis among the participants. Further research is needed to explore the potential underlying mechanisms and additional factors influencing the development and progression of seborrheic dermatitis.

Keywords: Stress level; seborrheic dermatitis; skin inflamation; human and health

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Highlights:
1. This research investigated a previously unexplored relationship between stress levels and seborrheic dermatitis in Indonesia.
2. It has been shown that a significant proportion of medical students are susceptible to sleep problems, which can negatively impact their academic performance and mental health.
3. This recent study found that seborrheic dermatitis affects men on average more frequently than women.

INTRODUCTION

Seborrheic dermatitis is a prevalent type of superficial inflammation with papulosquamous morphology that affects sebaceous gland-rich areas. This skin condition is characterized by a reddish color and flaky patches (Malak 2014, Enos et al. 2017, Elgash et al. 2019). This disease is a chronic condition that develops in adults and adolescents with variable degrees of severity. The symptoms of this skin condition may vanish briefly but reappear during stress (Borda et al. 2019, Piquero-Casals et al. 2019). Seborrheic dermatitis is a skin inflammation that can persist for years through recurrence and remission despite care and therapy. The face and scalp are the most affected sites, although it frequently involves multiple areas (Gary 2013, Sasseville 2018, Castillo et al. 2019).

The prevalence of seborrheic dermatitis is around 5% globally. However, nearly 50% of the world's population is affected by dandruff, a moderate and non-inflammatory form of seborrheic dermatitis (Tucker & Masood 2023). The prevalence of seborrheic dermatitis in Indonesia among the population within the age range of 12–20 years is 26.5%. This number exceeds the prevalence of seborrheic dermatitis in other Asian nations (Sahidah & Agustin 2018). Sebaceous gland activity, Malassezia colonization, epidermal barrier

integrity, host immunological response, neurogenic variables, emotional stress, and dietary factors contribute to an individual's vulnerability to acquire seborrheic dermatitis. In situations of depression and emotional stress, seborrheic dermatitis is more common. In addition, sleep deprivation and stress have been linked to the deterioration of seborrheic dermatitis (Borda & Wikramanayake 2015, Cheong et al. 2015).

A large percentage of medical students suffer from sleep disorders that have a severe effect on their academic performance and mental health. Long hours of lecture and study, clinical clerkships that include overnight duty, mental stress, lifestyle choices, and heavy use of social media, notably during the COVID-19 pandemic, contribute to the high prevalence of sleep problems among medical students (Turkmen et al. 2020, Perotta et al. 2021, Pendlebury et al. 2022). The Basic Health Research by the Minister of Health of the Republic of Indonesia (2018) estimated the prevalence of mental and emotional disorders in Indonesia to be 26 million, or 9.9%, among the population aged 15 and older. Research at an Indonesian medical school reported moderate stress levels in 57.23% of the students (Wahyudi et al. 2017). A comparable study conducted in Saudi Arabia found that the prevalence of severe stress was 33.8%. The condition was more prevalent among junior students (Saeed et al. 2016).

For medical students, chronic stress can be exhausting and lead to the gradual degradation of the immune system and individual health. Most medical students find it difficult to avoid stress, although chronic stress has a detrimental effect on their immune systems (Bekhbat & Neigh 2018, Perotta et al. 2021). It decreases the body's capability to fight against diseases and diminishes health. Chronic stress has also been shown to decrease the number and functionality of immune cells, which are essential to the body's anti-infection response (McEwen 2006, Saif et al. 2018). Therefore, the purpose of this study was to analyze the relationship between stress levels and seborrheic dermatitis among medical students, particularly the class of 2020, at Universitas Tarumanagara, Jakarta, Indonesia.

MATERIALS AND METHODS

This research was an analytical-observational study with a cross-sectional design. This study analyzed the relationship between the independent (i.e., stress levels) and the dependent (i.e., the case of seborrheic dermatitis) variables. The researchers observed the necessary variables in this study based on the specified subjects at the same time, though not all subjects had to be observed on the same day or at the same time (Spector 2019). The study was conducted at the Faculty of Medicine, Universitas Tarumanagara, Jakarta, Indonesia, from January to April 2022.

All medical students from the class of 2020 were chosen as the population of this study. In addition, the sample was collected using a simple random sampling technique (West 2016). The selection criteria for participation in this study were active undergraduate students from the class of 2020 at the Faculty of Medicine, Universitas Tarumanagara, who consented to participate in this study and signed the provided informed consent form. Subjects excluded from this study included those currently being treated for seborrheic dermatitis, those taking anti-stress medication, and those not willing to cooperate in completing the questionnaire. The sampling procedure resulted in a total of 114 participants.

The participants filled out a questionnaire, which was distributed online through Google Forms. The Depression, Anxiety, and Stress Scale (DASS-42) was used to construct the questionnaire to assess the stress levels of the participants (Habibi et al. 2017). The questionnaire results were summarized in scores according to the questionnaire answers. A score of 0–14 would indicate that the subject was at a normal stress level, while a score of 15–42 would indicate that the stress level was far from normal. Furthermore, the seborrheic dermatitis variable was assessed using a dermatological clinical evaluation that sought to quantify the magnitude of the skin condition according to the Seborrheic Dermatitis Area and Severity Index (SDASI) criteria (Emre et al. 2012). The data obtained were then analyzed using a cross-sectional assessment of the relationship between stress levels and seborrheic dermatitis. The evaluation was performed by considering both statuses at the same time (Wang & Cheng 2020).

This research utilized a questionnaire that is widely accepted and reliable for assessing similar cases. The DASS-42 measured the depression, anxiety, and stress levels comprehensively, providing a holistic view of the participants' psychological well-being (Makara-Studzińska et al. 2022). The DASS-42 questionnaire was distributed conveniently through Google Forms, facilitating efficient data collection. Similarly, the SDASI criteria offered a standardized method to evaluate the area and severity of seborrheic dermatitis (Cömert et al. 2013). The selection of these instruments aligned with the research objectives and previous studies, allowing for significant comparisons and contributing to existing knowledge.

The Chi-square test with a significance level (p) of 0.05 was employed in IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA) to process the acquired data and to determine the correlation between the two variables (McHugh 2013). The obtained p-value below 0.05 indicated a statistically significant relationship or correlation between the two variables. On the other hand, if the p-value was above 0.05, it suggested no statistically significant relationship between the variables. In the case of an observed association that happened by chance, any relationship between the variables might not be meaningful or generalizable to the population.

RESULTS

The total number of respondents or subjects in this study was 114. It was found that most of the research subjects who provided responses were female, with as many as 72 participants (63.2%). The age of the research subjects varied, with the largest proportion (69 individuals, 60.5%) being 19 years old (Table 1). In contrast, the age groups with the fewest respondents were those aged 21 and 22, with only four individuals in each group.

Table 1. Characteristics of the research subjects based on gender and age.

Characteristics	Frequency	%
Gender		
Female	72	63.2
Male	42	36.8
Age (years old)		
18	6	5.3
19	69	60.5
20	29	25.4
21	5	4.4
22	5	4.4

The number of participants with abnormal stress levels was greater (59 individuals, 51.8%) than those with normal stress levels (55 individuals, 48.2%). As many as 61 (53.5%) participants had seborrheic dermatitis, while 53 (46.5%) participants did not (Table 2).

Table 2. Distribution of the research subjects based on stress levels and seborrheic dermatitis cases.

Variables	Frequency	%
Stress level		
Normal	55	48.2
Abnormal	59	51.8
Seborrheic dermatitis		
Yes	61	53.5
No	53	46.5

It was found that the majority of participants who had seborrheic dermatitis experienced infrequent recurrences (44 individuals, 72.1%), but one participant suffered persistent symptoms (1.6%) (Table 3). Most participants with seborrheic dermatitis (86.9%) experienced mild complaints related to the skin condition, indicating manageable effects. Moderate complaints were reported by 11.5% of the participants, while only 1.6% reported severe complaints. Itching was the most commonly reported symptom, with 80.3% of the participants mentioning it. A small percentage (6.6%) reported a sensation of heat, while 13.1% did not report any specific complaints.

Table 3. Distribution of recurrence frequency, complaint severity, and symptoms of seborrheic dermatitis.

Seborrheic dermatitis	Frequency	%
Frequency of recurrence		
Seldom	44	72.1
Sometimes	12	19.7
Often	4	6.6
Always	1	1.6
Complaint severity		
Mild	53	86.9
Moderate	7	11.5
Severe	1	1.6
Symptom		
Itching	49	80.3
Burning sensation	4	6.6
Nothing	8	13.1

According to Table 4, women (58.3%) reported a higher percentage of stress occurrence compared to men (40.5%). This indicated that women in the study sample had a higher prevalence of stress. Among the age groups analyzed, individuals aged 18 had the lowest percentage of stress occurrence, with only two individuals (1.8%) reporting stress. Individuals in the 21-year-old age group had a slightly higher proportion, with three individuals (2.6%) experiencing stress. In contrast, individuals aged 20 showed a notable presence of stress, with 20 individuals (17.5%) reporting stress. The highest percentage of stress occurrences was observed among individuals aged 19, with 34 individuals (29.8%) experiencing stress. It is important to note that no occurrences of stress were reported among individuals aged 22, although this did not necessarily indicate a complete absence of stress among all individuals in that age group.

Table 4. Distribution of gender and age in the context of stress occurrences.

Variables	Stress occurrence	
	Yes (n, %)	No (n, %)
Gender*		
Men	17 (40.5)	25 (49.5)
Women	42 (58.3)	30 (41.7)
Age (years old)		
18	2 (1.8)	4 (3.5)
19	34 (29.8)	35 (30.7)
20	20 (17.5)	9 (7.9)
21	3 (2.6)	2 (1.8)
22	0 (0)	5 (4.4)

*Percentages for gender were calculated separately for men and women.

According to Table 5, it was found that the majority of the participants who had seborrheic dermatitis were in the 19-year-old age group. However, it corresponded to the large number of participants aged 19.

Table 5. Distribution of age in the context of seborrheic dermatitis occurrences.

Variable	Seborrheic dermatitis	
	Yes (n, %)	No (n, %)
Age (years old)		
18	2 (1.8)	4 (3.5)
19	39 (34.2)	30 (26.3)
20	16 (14)	13 (11.4)
21	1 (0.9)	4 (3.5)
22	3 (2.6)	2 (1.8)

Table 6 shows the relationship between gender and the occurrence of seborrheic dermatitis. It provides the number and percentage of individuals with and without seborrheic dermatitis. In the group of male participants, 24 individuals (57.1%) had seborrheic dermatitis, while 18 individuals (42.9%) did not have the condition. In the group of female participants, 37 individuals (51.4%) had seborrheic dermatitis, while 35 individuals (48.6%) did not. The Chi-square test results showed a relationship between gender and the incidence of seborrheic dermatitis (p=0.000). The likelihood of developing seborrheic dermatitis was significantly different between men and women in the study.

Table 6. Relationship between gender and the occurrence of seborrheic dermatitis.

Variables	Seborrheic dermatitis		p
	Yes (n, %)	No (n, %)	
Gender*			
Male	24 (57.1)	18 (42.9)	0.000
Female	37 (51.4)	35 (48.6)	

*Percentages for gender were calculated separately for males and females.

Table 7 demonstrates that the prevalence of seborrheic dermatitis was higher among the research participants with abnormal stress levels (33 individuals) than among those with normal stress levels (28 individuals). The results of the Chi-square test showed that there was no relationship between stress and the occurrence of seborrheic dermatitis (p=0.591).

Table 7. Relationship between stress and the occurrence of seborrheic dermatitis

Stress	Seborrheic dermatitis		p
	Yes (n, %)	No (n, %)	
Stress level			
Normal	28 (24.6)	27 (23.7)	0.591
Abnormal	33 (28.9)	26 (22.8)	

DISCUSSION

Stress levels and gender

Seborrheic dermatitis was more prevalent among women than men, indicating a relationship between gender and the frequency of cases (p=0.000). A study by Silvia et al. (2020) supported the findings of this study. The 2019 study was conducted on patients at the Dermatology and Venereology Department of Dr. H. Abdul Moeloek General Hospital, Bandar Lampung, Indonesia, with results indicating a correlation between gender and seborrheic dermatitis cases, but the male research subjects experienced the condition more frequently than the female research subjects. It aligns with the hypothesis that men and women have different skin characteristics, including differences in the number of hair follicles, sebaceous and sweat glands, and hormones. A higher concentration of the androgen hormone in men's skin results in increased perspiration and hair growth (Silvia et al. 2020).

Research by Malak (2014) from January to December 2015 at the Dermatology and Venereology Department of Prof. Dr. RD Kandou Hospital, Manado, Indonesia, yielded the same results. The study found that men (67%) were more prone to seborrheic dermatitis. However, Nabillah (2021) collected data on patients treated between 2016 and 2019 in the Dermatology and Venereology Department of Meuraxa General Hospital, Banda Aceh, Indonesia, and obtained different results. In the study, seborrheic dermatitis cases were more prevalent among female patients in 2016 (69:61) and 2017 (73:72), but more prevalent among male patients in 2018 (85:47) and 2019 (48:14).

Many variables can affect the varying results of these recent studies. On average, seborrheic

dermatitis affects men more frequently than women (Zander et al. 2019). The cause is the stimulation of higher male androgen hormone levels, which increases sebum production due to higher sebaceous gland activity. An increased sebum production can result in the expansion of *Malassezia* and seborrheic dermatitis. However, although previous studies found that genders significantly affected the prevalence of seborrheic dermatitis, there were still several confounding variables that might account for disparities in seborrheic dermatitis cases between genders. Since seborrheic dermatitis is a complex condition with various factors contributing to its etiology, its actual cause is uncertain (Borda & Wikramanayake 2015).

The results of this study determined that more subjects experienced stress above the normal limit (59 individuals) compared to those with normal or mild-to-moderate stress (55 individuals). The percentage of women who suffered from stress (58.3%) was higher than that of men (40.5%). Nasrani & Purnawati (2015) observed different results in yoga participants in Denpasar, Indonesia, with men having significantly higher normal stress levels than women (95.1% vs. 49.6%). In addition, it was found that gender affected the stress score by up to 22%. Men and women react in exceptionally different ways when faced with a dilemma. Women's brains tend to generate negative arousal in response to stressful problems. In contrast, men often embrace competition and view it as a source of good motivation.

Research by Kountul et al. (2018) on students at the Faculty of Public Health, Universitas Sam Ratulangi, Manado, Indonesia, found a correlation between gender and the students' stress levels, which supports this study's findings. It was found that 53.9% of the female students and only 34% of the male students experienced excessive stress. This was due to the vulnerability of the female participants to stress conditions regulated by oxytocin, estrogen, and other sex hormones. Men tend to be more experimental, whereas women tend to be more insecure and sensitive.

According to Graves et al. (2021), female students are also more prone to stress. Unlike men, women possess four coping techniques, i.e., self-distraction, emotional support, physical support, and venting feelings through complaining. Self-distraction has been the most common strategy because it often provides quick relief.

Stress levels and ages

The ages in this study were homogeneous (18–22 years old), given that this age range falls within the category of the beginning of maturity (18–25 years old). Consequently, the age range did not affect the prevalence of stress among the research participants. The findings of a study by (Herwandha & Prastuti 2021), carried out at a university in Malang, Indonesia, support this study's findings. The study found that age was not more significant than affection in determining the emotional maturity of the students.

It is necessary to note that the findings of this study contradicted those of Vallejo et al. (2018), who found that stress levels tend to decrease with age. The various intervals used to classify age ranges in the two studies may explain the discrepancy in the findings. Our investigation focused on a narrow age range of 18 to 22, whereas Vallejo et al. (2018) used broader age categories. This difference in age classification might have affected the observed correlations between age and stress levels.

Stress levels and seborrheic dermatitis

This study revealed that there was no relationship between stress levels and seborrheic dermatitis cases in medical students at Universitas Tarumanagara ($p=0.121$). Similar findings were reported in a study conducted by Sari (2019) on students at the Labschool Kebayoran Senior High School, Jakarta, Indonesia. In contrast, Marlina & Sinaga (2021) obtained different findings from the class of 2017 medical students at Universitas Sumatra Utara, Indonesia. They found that there was a significant correlation between stress levels and seborrheic dermatitis cases with *Pityriasis sicca* manifestations. Stress was one of the risk factors for seborrheic dermatitis, which could be caused by a number of variables.

The presence or absence of a correlation between stress levels and seborrheic dermatitis can vary across different studies. Several factors may explain this discrepancy, including methodological differences, the heterogeneous nature of seborrheic dermatitis, individual variations and confounding factors, the timing and duration of stress, and the influence of psychological factors (Sasseville 2018, Saif et al. 2018, Zander et al. 2019). Seborrheic dermatitis is a complex condition that depends on genetic, immune, lifestyle, and environmental factors. Therefore, the relationship between stress and seborrheic dermatitis is likely multifaceted and may not be consistently observed.

According to a number of studies that have been mentioned, stress may be a risk factor related to seborrheic dermatitis. The Global Burden of Skin Disease project reported that seborrheic dermatitis can also influence an individual's mental health, resulting in a lower quality of life due to increased worry (Urban et al. 2021). The body secretes

adrenaline and cortisol in response to intense or sustained stress. If the stress response is positive, the increase in these hormones is temporary, and their impact on the skin is low. However, if the stress response is negative, the increase in these hormones is persistent and will have a greater impact on the skin. Cortisol can increase sebum production, resulting in blackheads (Rao et al. 2021). In addition, the persistent stress-induced inflammatory response impairs the body's ability to heal damaged skin.

In a study conducted by Saif et al. (2018) on medical students at a Saudi Arabian university, it was found that chronic stress harmed the skin by generating neuroendocrine changes that impair the immune system. As a result of this condition, the skin can lose its ability to respond to environmental dangers. Furthermore, a specific immune system may misidentify chemicals that enter the body, leading to autoimmunity. Mast cells play a vital role in the body's exaggerated immunological response to stress and in the induction of neurogenic inflammation. Some skin diseases, such as seborrheic dermatitis, may be caused by abnormalities in the body's immune system.

A study by Gül et al. (2017) found that the prevalence of seborrheic dermatitis was higher in the group with the highest levels of stress, indicating that psychological stress could be related to skin diseases. Stress is more prevalent among educated individuals, and its negative impacts on the occurrence of diseases are more prevalent among them. Choe et al. (2018) also researched the relationship between psychological stress and skin health. They examined the effect of stress on the barrier function of the stratum corneum. In their study, stress hindered the regeneration of the skin barrier by up to seven days in both male and female rats. Administering diazepam and chlorpromazine is suggested to enhance skin barrier recovery. Stress reduction using pharmaceuticals can enhance stratum corneum formation. Changes in the microbiome of stressed individuals' skin alter the colonization rate of *Malassezia* species, hence promoting *Malassezia* infection. These may contribute to the worsening of seborrheic dermatitis in individuals with elevated stress levels.

Strength and limitations

This research benefits medical personnel and students as it provides findings on the prevalence of seborrheic dermatitis cases in conjunction with stress levels. Despite the data indicating an absence of a correlation between the variables, this study may inspire further research to elucidate the causes of seborrheic dermatitis cases among people in academia. This study provides data on the number of students who had seborrheic dermatitis, which can be a foundation for further investigation. However, this study has a limitation due to the restricted size of the sample, as it only included students from the Faculty of Medicine, which does not fully reflect the population in an academic setting.

CONCLUSION

A considerable number of medical students experienced stress, mostly at mild-to-moderate levels. Furthermore, seborrheic dermatitis was prevalent among the medical student population. However, this study did not find any significant relationship between stress levels and seborrheic dermatitis cases.

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Conflict of interest

None.

Ethical consideration

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Author contribution

E conceptualized the study, designed the methodology, conducted the investigation and formal analysis, and drafted the original manuscript. HD was responsible for the validation and supervision, the writing of reviews, the editing of the manuscript, the curation of data, and the administration of the study.

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Original Research Report

DETECTION OF KNOCKDOWN-RESISTANCE HOMOZYGOUS MUTANT C1534C USING ALLELE-SPECIFIC POLYMERASE CHAIN REACTION IN *Aedes albopictus* AND *Aedes aegypti*

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ABSTRACT

Dengue hemorrhagic fever (DHF) has been endemic in Indonesia for decades. Insecticides are necessary to manage the transmission of the dengue virus. However, prolonged use of insecticides can lead to insecticide resistance. This study aimed to examine the genotype of mosquitoes using the allele-specific polymerase chain reaction (ASPCR) method. The ASPCR method was chosen for genotype detection due to its high sensitivity, affordability, and ease of design. Five mosquitoes were collected from human habitation in four different areas of Surabaya, Indonesia, namely Kranggan, Ulul Azmi Mosque, Ploso, and Kalijudan. Among them, three samples were identified as *Aedes albopictus* (A1, A2, and A5) and two samples were identified as *Aedes aegypti* (A3 and A4). The frequency of resistant alleles was analyzed using the Hardy-Weinberg package in RStudio version 2023.03.1. This study revealed that two mosquitoes carried homozygous mutant alleles with a band of 113 bp and three mosquitoes carried homozygous wild-type alleles with a band of 93 bp. Cysteine-to-cysteine (C/C) mutations and phenylalanine-to-phenylalanine (F/F) mutations at codon 1534 were observed in *Aedes aegypti* and *Aedes albopictus* mosquitoes. The homozygous mutant alleles were found in Kranggan, Surabaya, Indonesia. Further research is required to assess insecticide resistance and knockdown resistance (*kdr*)-like mutation by collecting more representative samples from larger areas within the region of Surabaya. Nevertheless, this study can be used as a reference for vector control and early prevention of dengue fever.

Keywords: Dengue; *Aedes albopictus*; *Aedes aegypti*; knockdown resistance; allele-specific polymerase chain reaction (AS-PCR)

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Highlights:
1. This research analyzed a previously understudied subject in Surabaya, Indonesia, and discovered knockdown resistance (*kdr*) mutations in *Aedes albopictus*.
2. The findings of this study can prompt further research, including bioassay testing and the development of more potent insecticides.

INTRODUCTION

Aedes mosquitoes are carriers of several arboviruses that can infect humans. These mosquitoes cause illnesses such as chikungunya, yellow fever, zika, and dengue fever (Vu et al. 2020). Female *Aedes aegypti* mosquitoes are the primary vector responsible for spreading viruses to humans. On the other hand, female *Aedes albopictus* mosquitoes serve as the secondary vector (Balaska et al. 2020). Indonesia is quite well known for its tropical weather and high humidity. These factors greatly

contribute to the increased danger of mosquito-borne viral illnesses, including dengue hemorrhagic fever (Maula et al. 2018).

Aedes albopictus is the Latin name for mosquitoes with smaller physical characteristics and greater flying range than *Aedes aegypti*. *Aedes aegypti* and *Aedes albopictus* differ morphologically in the location of the back (mesonotum) (Rahayu & Ustiawan 2013). *Aedes aegypti* has a line-shaped back with two curved lines and two straight white lines, while *Aedes albopictus* has only one white stripe on the mesonotum. In terms of physical appearance, *Aedes albopictus* mosquitoes commonly have a darker color than *Aedes aegypti* (Dania 2016).

There are four distinct serotypes of dengue virus (DENV), i.e., DENV-1, DENV-2, DENV-3, and DENV-4. DENV infection can be asymptomatic, and the illness may manifest as dengue fever (DF), dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS) (Wardhani et al. 2017, Soegijanto et al. 2021). In Indonesia, dengue hemorrhagic fever was initially reported in Jakarta and Surabaya in 1968. Since then, cases have been increasing annually and spreading to new areas in Indonesia (Soegijanto et al. 2021). Currently, dengue cases have been reported in 34 Indonesian provinces. Surabaya had the highest number of cases of dengue virus infection in East Java during 2010–2013 (Wardhani et al. 2017, Putri et al. 2019).

The use of insecticides is essential to prevent the spread of DENV. People have been using insecticides to prevent the spread of diseases caused by dengue vectors, which have the potential to become endemic in many countries (Balaska et al. 2020). Initially, dichlorodiphenyltrichloroethane (DDT) and dieldrin were used to control dengue vectors in Indonesia. However, the use of these chemicals was terminated in 1970 because of the resistance developed in mosquitoes. Organophosphate and pyrethroid pesticides have been employed as alternatives to manage dengue vectors (Silalahi et al. 2022).

Insecticides that prevent dengue fever in Indonesia become less effective as mosquitoes develop resistance. Insects exposed to insecticides for a particular period produce insecticide-resistant offspring due to selection pressure across generations (Amelia-Yap et al. 2018). Resistance to pesticides in mosquitoes is referred to as knockdown resistance (*kdr*). Mutations in sodium channel genes cause a decrease in the sensitivity of these genes (Kushwah et al. 2015, Wuliandari et al. 2015). Resistance to pyrethroid pesticides in *Aedes albopictus* and *Aedes aegypti* has been observed. The first identification of a *kdr* mutation in *Aedes albopictus* was reported in Singapore in 2009. The investigation discovered that 24 of the 26 mosquitoes tested positive for F1534C mutation (Auteri et al. 2018).

Another study conducted in Makassar, Indonesia, found similar findings. The study revealed that a number of *Aedes aegypti* mosquitoes contained F1534F, F1534C, and C1534C mutations (Hamid et al. 2017). Mutations were detected in mosquitoes according to studies conducted in several countries, such as Malaysia, Costa Rica, Brazil, India, China, Japan, France, Italy, and the United States. Research conducted in a number of regions in Indonesia also yielded similar results (Fauziyah et al. 2021). In this study, the purpose was to determine whether the obtained samples carried mutations or not by employing the allele-specific polymerase chain reaction (AS-PCR) method.

MATERIALS AND METHODS

Adult mosquitoes (n=5) were collected from residential areas in Surabaya, Indonesia (Figure 1). Three mosquitoes were identified as *Aedes albopictus*, while the other two were identified as *Aedes aegypti*. The mosquito samples were labeled as A1, A2, A3, A4, and A5. The samples A1, A2, and A5 were *Aedes albopictus* mosquitoes, while the samples A3 and A4 were *Aedes aegypti* mosquitoes. The RNA from the samples A1–A5 was extracted using QIAamp® Viral RNA Kit (Qiagen, Germany) in accordance with the manufacturer's instructions (Setiawan et al. 2023).

Table 1. AS-PCR steps for detecting mutations in the samples.

Step	Temperature and time	Cycle
Initial denaturation	94 °C in 2 minutes	1
Denaturation	94 °C in 30 seconds	
Annealing	60 °C in 30 seconds	35
Extension	72 °C in 30 seconds	
Elongation	72 °C in 2 minutes	1

The mutant *kdr* allele was generated using an AS-PCR assay. The PCR reaction was carried out by referring to a study by Atencia et al. (2016). The volume of the PCR reaction was 22 µL, which contained 12.5 µL of green PCR master mix, 7.5 µL of nuclease-free water, 0.5 µL each of forward primers (Cys1534f and Phe1534f), and 1 µL of reverse primer (Cys1534r). The AS-PCR steps are presented in Table 1.

The use of primers in this study was based on a study by Atencia et al. (2016). A thermal cycler was used

to carry out a PCR reaction. The AS-PCR method was chosen because it can distinguish between homozygous and wild-type alleles. Furthermore, this method has advantages, such as being fast, inexpensive, highly sensitive, and easy to design (Setiawan et al. 2023).

Table 2. The oligonucleotide sequences utilized to amplify fragments of the FC gene.

Primer sequence		Product (bp)
Cys1534f	5'GCGGGCAGGGCG GCGGGGGCGGGGC CTCTACTTTGTGTT CTTCATCATGTG3'	113
Phe1534f	5'GCGGGCTCTACTT TGTGTTCTTCATCA TATT3'	93
Cys1534r	5'TCTGCTCGTTGAA GTTGTCGAT3'	

The first step in performing AS-PCR according to the guidelines was designing a specific primer to amplify different target regions between normal alleles (wild type) and mutant alleles. Specific primers must be designed to bind only to the mutated region of the mutant allele (Yang et al. 2017). The second step was optimizing PCR conditions, including temperature, time, and primary concentration, to obtain optimal results. These parameters were checked as needed during the initial trials and modifications (Lorenz 2012). The next step was electrophoresis and analysis of the results. Electrophoresis was performed following a PCR assay to separate the DNA fragments on an agarose gel. The DNA bands that emerged were then analyzed to determine the presence of mutant alleles (Lee et al. 2016).

It was important to note that the size and intensity of the band corresponded to a mutant or normal allele. The final step was interpreting the results based on the results of the DNA banding patterns and determining the presence of mutant and normal alleles in the tested samples (Mahdieh & Rabbani 2013). Observation of the differences in DNA banding patterns between the positive control, the negative control, and the tested samples is necessary for determining the AS-PCR result accurately.
According to Table 2, the F1534C gene could be associated with mutations in *Aedes* sp. mosquitoes and might cause resistance to permethrin-based insecticides. This mutation might occur at position 1534 in the gene encoding the targeted neurotoxin receptor (Fan & Scott 2020). AS-PCR was the laboratory method used to detect the presence of specific alleles in the DNA samples. In the context of F1534C, AS-PCR was used to identify the presence of F1534C mutation in *Aedes* sp. Mosquitoes (Darawi et al. 2013).

The AS-PCR method utilized specific primers designed to bind selectively to the normal (wild type) or mutational allele (F1534C) (Stenhouse et al. 2013). If a mutational allele was present, a primer designed for the mutational allele would amplify the DNA fragment. By using AS-PCR, researchers could detect the presence of the F1534C mutation in *Aedes* sp. mosquitoes by comparing the resulting DNA banding patterns to the positive and negative controls (Yang et al. 2017). This method could help monitor and research the spread of these mutations in mosquito populations and understand insecticide resistance. This method could also detect the mutation of the F1534C type in *Aedes* sp. mosquito gene.

In the study by Atencia et al. (2016), the different bands indicated the genotypes of *Aedes aegypti* and *Aedes albopictus*. If the band had appeared at 93 bp, the genotype was homozygous wild type (F/F or F1534F). If the band appeared at 113 bp, the genotype was homozygous mutant (C/C or C1534C). If the band appeared in two locations (93 bp and 113 bp), the sample was a heterozygous mutant. The frequency of the resistant alleles was analyzed using the Hardy-Weinberg package through RStudio version 2023.03.1+446.

Table 3. AS-PCR results and distribution of species according to sampling sites.

Sampling sites	*A. aegypti*	*A. albopictus*	Genotype			Allele frequency	
			SS (%)	SR (%)	RR (%)	S	R
Kranggan	0	2	0 (0)	0 (0)	2 (100)	0	1
Ploso	1	0	1 (100)	0 (0)	(0)	1	0
Ulul Azmi mosque	1	0	1 (100)	0 (0)	(0)	1	0
Kalijudan	0	1	1 (100)	0 (0)	(0)	1	0
Total	2	3	3	0	2		

SS: F1534F (susceptible allele/homozygous wild type); SR: F1534C (susceptible resistant/heterozygous mutant); RR: C1534C (resistant resistant/homozygous mutant).

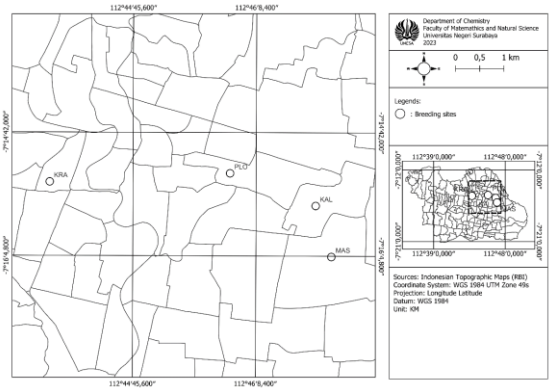

Figure 1. Geographical map of Surabaya, with a focus on four breeding sites (KRA: Kranggan; PLO: Ploso; MAS: Ulul Azmi Mosque, Universitas Airlangga; KAL: Kalijudan). QGIS version 3.26.3 was used to create this figure.

RESULTS

A total of five samples, which consisted of three *Aedes albopictus* and two *Aedes aegypti* mosquitoes, were tested. These two species of mosquitoes had differences in the dorsal mesonotum that could be detected with the naked eye. The thorax of the *Aedes aegypti* samples had a white curving line and two short white lines in the middle. On the other hand, the *Aedes albopictus* samples had a thorax with only a white stripe. In addition, the color of the *Aedes albopictus* samples was darker than that of the *Aedes aegypti* samples.

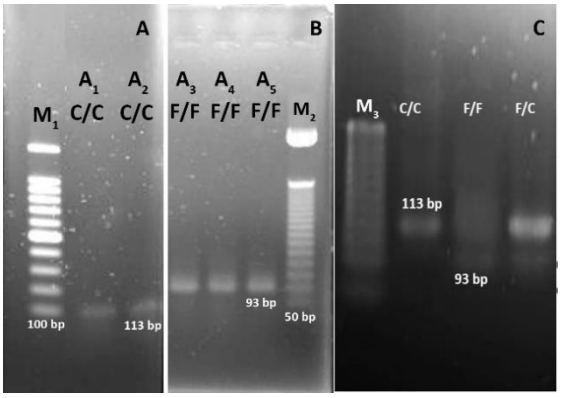

Figure 2. The electrophoresis results of the PCR, with (A) and (B) representing the study results and (C) representing the reference figure.
M_1=100 bp marker; M_2=50 bp marker; M_3=25 bp marker; C/C=homozygous mutant; F/F=homozygous wild type; F/C=heterozygous mutant.

Different bands from the analysis results indicated the *Aedes aegypti* and *Aedes albopictus* genotypes. The genotype was homozygous wild type (F/F or F1534F) if the band appeared at 93 bp, while it was homozygous mutant (C/C or C1534C) if the band appeared at 113 bp. On the other hand, the genotype was heterozygous mutant if the band was present in two locations (93 bp and 113 bp). The results of this study are presented in Figure 2. The figure showed that two samples contained point mutations. In contrast, the other three samples did not carry any mutations. Two samples (A1 and A2) tested positive for the homozygous C1534C mutation, while the other three samples (A3, A4, and A5) tested positive for the homozygous wild type F1534F.

Table 3 presents the distribution of species and allele frequencies. Two samples from Kranggan (C/C frequency=100%) and one from Ploso (F/F frequency=100%) were *Aedes albopictus*. The remaining samples from Ulul Azmi Mosque and Kalijudan were *Aedes aegypti* and had the genotype F/F (100%). Heterozygous mutants (F/C) were not detected in all areas. The total frequency of the F allele was 100%, while the frequency of the C allele was also 100%.

The provided data offered an overview of the AS-PCR results for the C1534C and F1534F mutations in the mosquito samples that were analyzed. This research's findings would help to improve our understanding of the presence and distribution of these mutations in *Aedes albopictus* and *Aedes aegypti* mosquito populations.

DISCUSSION

This article highlights the detection of the *kdr* mutant allele in mosquito samples collected from the study sites. The use of the AS-PCR method in this study was in line with previous studies by Stenhouse et al. (2013) and Lee et al. (2016), which also utilized the same method to identify mutant alleles in mosquitoes. This method can provide rapid, accurate, and cost-effective genotyping results.

A previous study by Fauziyah et al. (2021) also examined samples of *Aedes aegypti* from several sites by extracting RNA from these mosquitoes and analyzing the point mutations. In this study, two bands appeared at 113 bp, indicating that the samples carried homozygous mutations. On the other hand, three bands appeared at 93 bp, indicating that the samples were homozygous wild type. The percentage of homozygous mutant in the samples collected from Kranggan was 100%. Meanwhile, the percentage of homozygous wild-type allele in samples collected from Ploso, Ulul Azmi Mosque, and Kalijudan was also 100%. There was no heterozygous mutant (F/C) detected in the samples. A heterozygous mutant is the product of a genetic mutation in the neurotoxin target receptor gene of *Aedes* sp. mosquitoes. Changes in the amino acid phenylalanine (F) to cysteine (C) at position 1534

are the cause of this mutation (Zhu et al. 2019). It results in the presence of two distinct alleles, a wild-type allele with the amino acid phenylalanine (F) and a mutant allele with the amino acid cysteine (C), at position 1534 within the same gene (Saingamsook et al. 2017). Therefore, the F-to-C mutation at position 1534 can be referred to as F/C, which indicates a variation of the normal and mutant alleles at that position.

A study by Zheng et al. (2022) examined the pattern of *kdr* mutations in mosquitoes collected from several districts in Guangzhou, China. The results showed that homozygous F1534F wild-type mutations were found in 15 field populations of *Aedes albopictus* collected from 11 districts. Four alleles were identified at codon 1534 of domain III, i.e., F/C, F/F, F/L, and F/S. These alleles corresponded to the mutant alleles F1534C, F1534L, and F1534S, as well as the wild-type allele F1534F. A wild homozygous F/F (TTC/TTC) genotype was observed, and two cases of CTC were found in Conghua district. TTG was the primary base sequence of the F1534L allele (Zheng et al. 2022). Another study by Hamid et al. (2017) performed F1534C allele-specific PCR and confirmed F to C point mutation spread in domain III voltage-gated sodium channel (VGSC) genes. The homozygous CC and FC heterozygous frequencies in the resistant phenotypes were found to be 0.21 and 0.25, respectively.

In India, a novel T1520I point mutation with a frequency of 0.13 and an F1534C mutation with a frequency between 0.40 and 0.79 have been identified (Kushwah et al. 2015). The F1534C *kdr* mutation in susceptible and phenotypically resistant mosquito specimens was further discovered using 200 successful amplifications and partial sequencing of the VGSC genes. In northern West Bengal, India, the population under study consisted of 81% homozygous (1534F/F), 12.5% heterozygous (1534F/C), and 6% homozygous (1534C/C) samples carrying the F1534C *kdr* mutation (Modak & Saha 2022). On the other hand, samples of sensitized (dead) mosquitoes showed C/C mutations with a frequency of 0.13. While the C-containing allele was more common in heterozygotes, the CC allele was relatively rare in the sensitive sample group. In Denpasar, Indonesia, the overall frequency of C in mosquitoes was lower than in the F of Domain III sequences, of which the database was collected in GenBank. The F1534C mutations and the F1534F wild types were found, with accessions no. KY078304 and KY078303 (Hamid et al. 2017).

Selection pressure on mosquito vector insecticide target sites has been reported and subsequently detected globally in the principal mosquito vector species, i.e., the *Aedes* mosquitoes. It results in the incidence and evolution of mutations at one or more locations in the relevant gene (Sokhna et al. 2013). Multiple studies have demonstrated that mutations impact the sensitivity of mosquitoes to a variety of insecticides. The presence of *kdr* mutations poses a risk of pyrethroid and DDT resistance (Smith et al. 2018, Chen et al. 2019). There are two primary methods that mosquito vectors have developed to resist the lethal impact of insecticides. These methods involve either an increase in the insensitivity of insecticide target sites caused by genetic point mutations or an increase in the activity of enzymes responsible for detoxifying insecticides (Liu 2015). Understanding the molecular mechanisms behind pesticide resistance can help prevent the spread of dengue fever. Pyrethroid insecticides, commonly used in spraying or fogging, can disrupt the central and peripheral nervous systems of mosquitoes or insects. The disruptions may cause convulsions, paralysis, and death (Davies & Williamson 2009). However, the number of mosquitoes increases instead as a result of the inability to manage pyrethroid-resistant mosquitoes.

Strength and limitations

Due to the limited number of samples collected from only a few areas in Surabaya, Indonesia, the genotype variation in the population could not be represented. Further research is required to collect more representative samples from more areas in the Surabaya region and to investigate the *kdr* mutation. However, this study remains significant as a prompt for *kdr* mutation detection and the development of new vector management strategies.

CONCLUSION

Homozygous mutant alleles were identified in *Aedes albopictus* mosquitoes collected from Kranggan, Surabaya, Indonesia. The insecticide resistance status needs to be assessed, and new vector control strategies should be implemented accordingly. It is anticipated that the findings of this study will serve as a reference for future research and contribute to the development of synthetic or natural insecticides that are more effective for dengue vector control.

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Conflict of interest

None.

Ethical consideration

This research was authorized by the Institute for Research and Community Service of Universitas Airlangga, Surabaya, Indonesia, with the approval No. 24-934/UN3.14/PPd/2013 on 20/8/2013.

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Author contribution

SZF was responsible for manuscript preparation as well as data collection and analysis. ARS was responsible for manuscript preparation, data collection, and grammar checking. SF was responsible for manuscript preparation and data confirmation. SM and ECD were responsible for manuscript preparation and data collection. SWN collected the data and drafted the manuscript. SEC validated the data and contributed to the preparation of the manuscript. TT was responsible for data validation and manuscript preparation. THS was responsible for the conceptualization, data collection, and investigation.

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Original Research Report

COVID-19 DISEASE SEVERITY AND BLOOD TEST RESULTS IN UNVACCINATED AND VACCINATED PATIENTS AT BHAYANGKARA HOSPITAL, DENPASAR, INDONESIA

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ABSTRACT

The COVID-19 pandemic caused by SARS-CoV-2 is an unprecedented event in human history. Vaccines are a safe, long-term solution for addressing the COVID-19 pandemic. This study aimed to investigate the differences in disease severity and blood test results between unvaccinated and vaccinated COVID-19 patients. This study used an analytical observational method with purposive sampling. A total of 90 COVID-19 patients at Bhayangkara Hospital, Denpasar, Indonesia, were divided into three groups: unvaccinated group (V0), two-dose vaccinated group (Vp), and three-dose vaccinated group (Vb). Primary data were collected from July to December 2022, while secondary data were collected from January 2021 to June 2022. The data were analyzed using the Kruskal-Wallis test followed by the Mann-Whitney test, as well as one-way ANOVA test followed by Tukey's honestly significant difference (HSD) test with a confidence interval (CI) of 95% and α of 5%. The results revealed significant differences in disease severity ($p<0.001$). V0 had a higher percentage of severe (36.7%) and critical (6.7%) symptoms than Vp (severe=10.0%; critical, n=0) and Vb (severe and critical, n=0). The follow-up tests revealed significant differences in disease severity between V0 and Vp ($p<0.001$), V0 and Vb ($p<0.001$), as well as Vp and Vb ($p=0.001$). Blood test results revealed significant differences in lymphocytes ($p=0.005$), monocytes ($p<0.001$), monocyte-to-lymphocyte ratio (MLR) ($p<0.001$), and eosinophils ($p=0.037$). The follow-up tests revealed significant differences in these four indicators between V0 and Vb, in all parameters except for lymphocytes between V0 and Vp, and in lymphocytes only between Vp vs Vb. In conclusion, unvaccinated patients had a higher percentage of severe and critical symptoms than vaccinated patients. The blood test results revealed significant differences in lymphocytes, monocytes, MLR, and eosinophils. Unvaccinated patients had lower lymphocyte counts, higher MLR levels, and higher monocyte counts than vaccinated patients.

Keywords: COVID-19; SARS-CoV-2; vaccine, disease severity; blood test results; public health

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Highlights:
1. This original research report on the differences in disease severity and blood test results between unvaccinated and vaccinated COVID-19 patients is quite novel, as the COVID-19 pandemic was still happening when this study was carried out.
2. This original research report offers information on the advantages of the COVID-19 vaccines and provides data that the public may use as scientific evidence to counter hoaxes.

INTRODUCTION

Disease severity in COVID-19 is often related to the proportion of immune cells in the patient's body. In COVID-19 patients with severe inflammation, there is an increase in the inflammatory response and a decrease in the immune response. These responses are shown in the blood test results, including the leukocyte count, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR) (Ok et al. 2021, Erdogan et al. 2021). Vaccination is necessary

for COVID-19 management to reduce the number of COVID-19 cases. The COVID-19 vaccines were developed with the objective of forming individual immunity to accelerate the emergence of herd immunity (Saija 2021).

The Indonesian government aimed to get 234.67 million people vaccinated with a complete or primary dose (two-dose vaccination) and a booster. However, according to a report from the Indonesian Ministry of Health, 74.27% of the targeted population was fully vaccinated, and only 28.57% had received a booster shot as of early December 2022 (Indonesian COVID-19 Task Force 2022). A study by Hartono et al. (2022) showed that the number of hoaxes regarding vaccines was known to contribute to low public confidence in the COVID-19 vaccines. Therefore, this study aimed to find scientific evidence about the benefits of COVID-19 vaccines to dismiss hoaxes.

MATERIALS AND METHODS

This paper presents an analytical observational study conducted at Bhayangkara Hospital, Denpasar, Indonesia. The methods for this study were adapted from prior research with modifications (Hanafi et al. 2021, Islas-Vazquez et al. 2022). This study used a purposive sampling technique. COVID-19 patients, who were infected for the first time, older than 18 years, free of comorbid diseases, not pregnant, and had blood tests within 24 hours of hospital admission, served as the samples. Primary data were collected by direct observation of patients during July–December 2022, while secondary data were gathered from the patients' medical records between January 2021 and June 2022.

The samples were divided into three groups: V0 (unvaccinated), Vp (two-dose vaccinated), and Vb (three-dose vaccinated). This categorization followed the methodology of a study by Bakasis et al. (2022). Each group consisted of subjects randomly selected from as many as 30 patients. The patients' disease severity and blood test results were evaluated. The severity of the COVID-19 disease were categorized as asymptomatic, mild, moderate, severe, and critical (Hanafi et al. 2021). In addition, blood tests were used to analyze the erythrocyte, hemoglobin, hematocrit, leukocyte, neutrophil, lymphocyte, monocyte, eosinophil, basophil, platelet, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), creatinine, and blood urea nitrogen-to-creatinine ratio (BCR) (Mao et al. 2021). Direct observation provided information on the severity of the patients' diseases. The information was then compared to the criteria in the COVID-19 guidelines issued by the Indonesian Ministry of Health (Indonesian COVID-19 Task Force 2022). Blood samples were analyzed in the laboratory of Bhayangkara Hospital, Denpasar, Indonesia. Blood sampling was performed by puncturing superficial veins with a syringe. The blood samples were placed in edetic acid (EDTA) tubes, rested at room temperature for at least 20 minutes, and homogenized for 5–10 minutes with a roller mixer. After inserting the samples into the Swelab Lumi hematology analyzer, the printed results were used to verify the analysis.

The data were analyzed using a One-Way ANOVA test with a Tukey HSD follow-up test to determine if there was a normal distribution and homogeneous variance. However, if the data were not normally distributed and had a non-homogenous variance, analysis would be performed using the Kruskal-Wallis test with the Mann-Whitney follow-up test (Cleophas & Zwinderman 2016, Lee 2022). All statistical tests were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA), with a 95% confidence level and a 5% tolerable error. A result of $p<0.05$ was considered significant.

RESULTS

This study involved 90 COVID-19 patients hospitalized at the Bhayangkara Hospital, Denpasar, Indonesia. The patients consisted of 52 men (58%) and 38 women (42%). Mild COVID-19 patients were those having specific symptoms (upper respiratory infection symptoms) and unspecific symptoms (e.g., anosmia, ageusia, indigestion) without any signs of hypoxia. On the other hand, moderate COVID-19 patients were those with pneumonic symptoms and SpO2>93% while breathing room air. Severe COVID-19 patients were those having pneumonic symptoms with a respiration rate of >30 breaths/minute, severe respiratory distress syndrome, or SpO2<93% while breathing room air. Critical COVID-19 patients were those with acute respiratory distress syndrome (ARDS), sepsis, septic shock, or other conditions requiring life support such as mechanical ventilation or vasopressor therapy.

After classifying the patients according to their disease severity, the total number of patients in each group was counted. Statistical analyses were conducted for each group. The obtained results are shown in Tables 1 and 2.

As shown in Table 1, the booster-vaccinated group had the highest percentage of mild symptoms with

no severe or critical symptoms. Critical COVID-19 symptoms were only found in V0, and severe COVID-19 symptoms were found in V0 and Vp.

Tabel 1. The results of the Kruskal-Wallis test analysis of the differences in disease severity.

Severity	Groups			p
	V0 (n=30)	Vp (n=30)	Vb (n=30)	
Mild	2 (6.7%)	11 (36.7%)	24 (80%)	<0.001
Moderate	15 (50.0%)	16 (53.3%)	6 (20%)	
Severe	11 (36.7%)	3 (10.0%)	0 (0%)	
Critical	2 (6.7%)	0 (0%)	0 (0%)	

Tabel 2. The results of the Mann-Whitney test analysis of the differences in disease severity.

	V0 vs Vp	V0 vs Vb	Vp vs Vb
Disease severity	<0.001	<0.001	0.001

Table 3. Mean differences from the analysis of blood test results with the Kruskal-Wallis test.

Parameters	Groups			p
	V0 (n=30)	Vp (n=30)	Vb (n=30)	
Hb	14.44±1.98	13.97±1.69	14.43±1.40	0.283
Leukocyte	7.63±2.94	6.72±2.64	7.26±1.90	0.248
Neutrophil	5.30±2.73	4.70±2.67	5.03±1.65	0.399
Lymphocyte	1.31±0.58	1.52±0.59	1.76±0.51	0.005
NLR	4.73±2.89	3.58±2.57	3.02±1.13	0.056
Monocyte	0.87±0.45	0.47±0.42	0.48±0.28	<0.001
MLR	0.82±0.69	0.35±0.33	0.28±0.15	<0.001
Eosinophil	0.09±0.10	0.13±0.09	0.22±0.38	0.037
Basophil	0.04±0.05	0.05±0.07	0.03±0.02	0.629
Platelet	196.00±59.97	232.10±76.86	222.07±45.66	0.142
PLR	173.29±76.60	171.75±78.50	137.46±52.56	0.090
Hematocrit	39.70±5.41	40.85±5.05	42.36±4.04	0.112
SGOT	40.57±30.15	40.27±32.00	32.67±16.42	0.518
SGPT	40.83±27.67	43.63±46.50	33.23±20.55	0.384
Urea	22.70±7.81	21.67±7.25	22.30±6.56	0.345
Creatinine	1.02±0.21	0.95±0.28	0.99±0.22	0.398

Table 2 shows the results of the follow-up test. There were significant differences in disease severity between V0 vs. Vp, V0 vs. Vb, and Vp vs. Vb. In addition, the mean results of the blood tests were analyzed statistically for those three groups. Table 3 shows the average blood test result in each group according to a number of parameters and the p-value for each parameter. The data obtained from the parameters shown in Table 3 were not normally distributed and had a non-homogenous variance. As shown in Table 5, analyses using the Kruskal-Wallis and Mann-Whitney tests were necessary for the follow-up tests.

Two parameters shown in Table 4 were normally distributed and had homogeneous variance. A One-Way ANOVA test was conducted for these parameters, and there were no significant differences in the results. Therefore, no follow-up test was conducted.

Table 4. Mean differences from the analysis of blood test results with One-Way ANOVA test.

Parameters	Groups		
	V0 (n=30)	Vp (n=30)	Vb (n=30)
Erythrocyte	4.81±0.62[a]	4.92±0.60[a]	4.93±0.58[a]
BCR	23.03±9.13[a]	27.89±25.52[a]	23.40±7.99[a]

Note: values with the same superscript ([a]) were not significantly different.

The blood test results for lymphocytes, monocytes, MLR, and eosinophils among the unvaccinated, 2-dose vaccinated, and 3-dose vaccinated groups were significantly different (p=0.005; p<0.001; p<0.001, and p=0.037, respectivelyOn the other hand, the other parameters showed no significant differences in the blood test results (p>0.05). There was a trend of decreasing lymphocytes and eosinophils as well as increasing MLR and monocytes in the unvaccinated group compared to the vaccinated group (Table 3). The follow-up test results for parameters with significantly different results (p<0.05) are shown in Table 5.

Tabel 5. Mean differences from the analysis of lymphocyte, monocyte, MLR, and eosinophil parameters with the Mann-Whitney test.

	V0 vs Vp	V0 vs Vb	Vp vs Vb
Lymphocyte	0.192	0.002	0.031
Monocyte	<0.001	<0.001	0.466
MLR	<0.001	<0.001	0.679
Eosinophil	0.047	0.016	0.706

Table 5 shows that the monocyte, MLR, and eosinophil parameters between V0 and Vp were significantly different, while the lymphocyte parameter was not. All indicators between V0 and Vb were significantly different. The lymphocyte parameter between Vp and Vb was significantly different, while the others were not.

DISCUSSION

Disease severity indicates the severity of the symptoms experienced by the patient. In this research, unvaccinated patients were more likely to experience moderate, severe, or critical symptoms compared to vaccinated patients. This result is in line with the research conducted by Antonelli et al. (2022). A significant difference in disease severity was caused by specific immunity against the COVID-19 infection in the vaccinated patients.

Lymphocyte count in the unvaccinated COVID-19 patients was lower compared to the vaccinated patients, as shown in Table 3. Lymphopenia, which is defined as a lymphocyte count <1.5x109/L, is common in severe or critical COVID-19 patients (Zhao et al. 2020, Zhang et al. 2020). It occurs due to the binding of Fas ligand (FasL) and Fas receptors (FasR) on damaged cells. The damaged cells can induce programmed cell death, thus decreasing blood lymphocyte levels (Mazzoni et al. 2020). Infections or other conditions affecting the blood can cause a decrease in the concentration of lymphocytes in the blood. The COVID-19 infection depletes and inhibits the expansion of T lymphocyte cells (Diao et al. 2020, Ouyang et al. 2020). It is possible for SARS-CoV-2 to cause infection in vitro because, although in a low level, human T cells also contain mRNA of the ACE2 receptor (Tavakolpour et al. 2020). The sequestration and infiltration of peripheral T lymphocytes can also cause lymphopenia. Lungs, digestive tract, and lymphatic tissue are some of the organs that have the potential to be affected (Huang & Pranata 2020). In this research, lymphopenia was most prevalent in the unvaccinated group, which had a higher percentage of severe and critical symptoms than the vaccinated group. These findings are in line with those of a prior study conducted by Huang & Pranata (2020). The study showed that vaccinated patients who had already developed an adaptive immune system were less susceptible to lymphopenia than unvaccinated patients.

In this study, there was a significant difference in monocyte counts. The unvaccinated group was more likely to experience monocytosis, an increase in monocytes in the blood >0.6x10^9/L (Mao et al. 2021). In severe SARS-CoV-2 infections, lung damage has been reported as increased numbers of monocytes and decreased numbers of lymphocytes in the blood. In the previous study of the blood immunity characteristics of COVID-19 patients, it was found that there was an increase in pro-inflammatory monocytes and IL-6 levels in severe and critical COVID-19 patients (Zhou et al. 2020). Another previous study showed that more than 50% of COVID-19 patients had monocytosis. Patients with severe or critical COVID-19 symptoms have an even higher risk of developing monocytosis (Mao et al. 2021, Porto et al. 2022). It is in line with the results of this study that showed the unvaccinated group had the highest percentage of severe and critical symptoms, thereby having a higher risk of experiencing monocytosis. In unvaccinated patients, adaptive immunity has not been formed optimally, and there is an excessive inflammatory response characterized by an increase in pro-inflammatory cytokines that causes cytokine storms (Zhou et al. 2020).

Referring to the two parameters above (lymphocytes and monocytes), the MLR indicator also had significant differences in this research. MLR is an inflammatory marker used to predict the severity of COVID-19 (Citu et al. 2022). MLR has a specificity of 90% for distinguishing COVID-19 patients from healthy people. In addition, MLR has a sensitivity of 75.79% for identifying the differences (Peng et al. 2020). Previous studies revealed that the MLR value in severe-critical COVID-19 patients typically increased significantly in comparison to mild-moderate COVID-19 patients. The MLR value was much higher in patients who were dying (Sun et al. 2020, Ertekin et al. 2021). The Mann-Whitney follow-up test revealed that the unvaccinated group in this research had the highest percentage of severe and critical symptoms as well as the highest MLR value.

Another parameter that showed a significant difference in the results of this study was eosinophils. Eosinophils were originally identified as the main effectors of allergies, but now these cells have been shown to have antiviral activity and function to enhance the immune response and suppress inflammation (Lindsley et al. 2020, Hernaningsih 2021). In spite of the significant difference, the values of eosinophils in all groups were in the normal range. However, the absence of eosinophils could be used to identify COVID-19 cases, especially those that have a higher probability of death, so that clinicians can start therapy earlier (Tanni et al. 2020).

Strength and limitations

The samples in this study were collected in almost similar conditions, thereby reducing research bias. The results of the study clearly illustrated the differences in disease severity and blood test results between unvaccinated and vaccinated patients. Therefore, the information in this paper can provide a scientific picture of the importance of COVID-19 vaccination for the public. However, the limitation of this research was that the number of research samples was limited. This was a unicentric study conducted in one hospital and covered only a small geographic area.

Due to the limitations of this research, further research is needed with a larger and more diverse number of samples and considering comorbid diseases. Comprehensive research also needs to be carried out by adding research variables (such as the type of vaccine) and data on anti-COVID antibody levels. This research still provides a potential opportunity in terms of knowledge and data that can be used as scientific information for the public.

CONCLUSION

Unvaccinated patients had a higher percentage of severe and critical symptoms than vaccinated patients. In addition, the number of lymphocyte counts in the unvaccinated group were lower, while the MLR levels and the number of monocyte counts were higher in the vaccinated group. However, the number of eosinophils in all groups was still within normal limits.

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Conflict of interest

None.

Ethical consideration

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Author contribution

CLM conceptualized the study, wrote and prepared the original draft, and collected the data. NIW conceptualized the study, developed the methodology, and reviewed and edited the manuscript. AASAS provided validation for the study. All authors have read and agreed to the final version of the manuscript for publication.

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Original Research Report

THE EFFECT OF CABBAGE (*Brassica oleracea* var. *capitata* L.) EXTRACT ON MACROPHAGE AND BLOOD VESSEL COUNTS IN CLEAN WOUND TISSUE OF MALE RATS (*Rattus norvegicus*)

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ABSTRACT

Cabbage is a widely consumed vegetable known for its health benefits due to its rich nutrients and phytochemicals, especially phenolic compounds, which are known to have potent anti-inflammatory and antioxidant effects. This study aimed to investigate the effects of cabbage extract on wound healing by observing inflammatory responses in wound tissue. A total of 24 male rats were divided into four groups, with six rats in each group. The experiment was conducted for five days by administering cabbage extract to the treatment group and distilled water to the control group. Skin wound tissues were collected from all rats for histological observation by counting the number of macrophages and blood vessels. The results of the observation were analyzed statistically using an independent sample t-test with $p<0.05$. The results showed that the number of macrophages and blood vessels in the treatment group was significantly higher than the control group on the third day and significantly lower on the fifth day. In conclusion, the administration of cabbage extract can accelerate the inflammatory and proliferative phases of wound healing by promoting the migration of cells, including macrophages, resulting in accelerated angiogenesis. In addition, the decreased number of macrophages and blood vessels during the proliferative phase showed that the healing phase had reached a more advanced stage.

Keywords: Healthy lifestyle; anti-inflammation; cabbage extract; wound healing

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Highlights:
1. Cabbage extract application accelerates wound healing and has an anti-inflammatory effect.
2. Cabbage extract serves as an affordable source material for wound healing and anti-inflammation.

INTRODUCTION

A wound can be defined as any disturbance in the normal anatomical structure of the body's tissues, from subcutaneous tissue to deeper tissues such as tendons, muscles, nerves, and bones, which is usually accompanied by the tearing of blood vessels. Under certain conditions, the wound may fail the normal healing process which leads to the development of chronic wound (Abdurrahmat 2014, Chhabra et al. 2017). A wound in the chronic stage significantly reduces the patient's quality of life. It can cause permanent disability, immobility, and pain. It also requires continuous treatment, which results in an increase in economic burden (Šitum et al. 2014). Early wound care is necessary because it helps accelerate wound healing and prevent the development of chronic wounds. Anti-inflammatory agents are usually used by the general public for wound care. The use of anti-inflammatory agents from natural products can be a solution because they have minimal side effects, easily available ingredients, and an effective cost (Sumayyah & Salsabila 2017).

During the last three decades, there has been a rapid

increase in the use of medicinal products and herbal supplements, with around 80% of people worldwide relying on herbal products as part of their primary health care. Apart from cultural beliefs, the use of plants as medicine is also supported by several studies, which have proved that various plants are known to contain a large number of bioactive compounds that provide various health benefits (Ekor 2014, Panche et al. 2016). Indonesia is a country with abundant natural resources due to the diversity in climate and geographical conditions that allow various types of plants to grow. It is possible for Indonesian people to take advantage of the various plants around them to meet various daily needs, one of which is medicine. Cabbage (*Brassica oleracea* var. *capitata* L.) is one of the plants that has the potential to be used as medicine. This plant is not only easily found and consumed in Indonesia but also all over the world. Cabbage contains various phytochemical compounds such as sulforaphane, flavonoids, glucosinolates, anthocyanins, phenolic acids, kaempferol, quercetin, and several other compounds that provide many benefits for improving a healthy lifestyle by acting as anti-inflammatory, antioxidant, antiviral, and antibacterial agents (Park et al. 2014, Lee et al. 2018).

The wound healing process until the integrity of the skin can return to its original state consists of three phases, i.e., inflammation, proliferation, and remodeling. The inflammatory phase consists of hemostasis and phagocytosis processes. Hemoestasis is the formation of a temporary wound matrix that functions to stop the bleeding, while phagocytosis is the process of cleaning the wound from the remnants of damaged tissue, pathogenic microorganisms, and apoptotic neutrophil cells (Krzyszczyk et al. 2018, Winarni et al. 2022). In the proliferative phase, granulation tissue is formed, replacing a temporary matrix with a new and stronger matrix. This matrix consists of fibroblasts, macrophages, and blood vessels. The last phase of the wound healing process is the remodeling phase, known as extracellular matrix maturation, which functions to maximize the structural integrity of the new tissue (Gonzalez et al. 2016, Landén et al. 2016).

In the healing of wounds, macrophages play a significant and constant role during the inflammatory, proliferative, and remodeling phases. When the inflammation subsides, the number of proinflammatory macrophages will be reduced, while the anti-inflammatory macrophages will be activated to regulate the process of tissue formation, including vascularization. Macrophages influence the formation of new vasculature because macrophages produce growth factors, including proangiogenic growth factors such as platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), and transforming growth factor beta (TGF-β) (Cañedo-Dorantes & Cañedo-Ayala 2019). This study was conducted to determine the effect of cabbage extract on the wound healing process by measuring the degree of inflammation through macrophages and blood vessel counts in the granulation tissue. The lower degree of inflammation indicates that wound healing occurs more rapidly (Rosique et al. 2015).

MATERIALS AND METHODS

This study was an experimental analytic study with a randomized posttest-only control group design. This study was conducted in the Biochemistry Laboratory, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. The research tools were cages, food, drinking water, a set of sterile surgical instruments to obtain the rats' wounded tissues, a 10% formalin buffer solution, and a light microscope. The materials included the experimental animals (*Rattus norvegicus*), ketamine and xylazine for anesthesia, distilled water, and 100% cabbage extract obtained from the Herbal Materia Medica Laboratory, Batu, Indonesia. The cabbage extract was produced by pharmacists at the Herbal Materia Medica Laboratory using a maceration method. A total of 3 kg of fresh cabbage was added to 96% ethanol as a solvent, then mashed with a blender and soaked in a glass container for 3x24 hours (Bhuana et al. 2021). The filtrate and the pulp were separated using a filter cloth, and the filtrate was evaporated until the solvent did not drip into the collecting flask. The result obtained was 100% cabbage extract in liquid form with a slightly thick consistency.

Experimental animals in this study were white male rats (*Rattus norvegicus*) obtained from the Biochemistry Laboratory, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. The criteria for the experimental animals in this study were male rats aged 2–4 months, with a weight of 130–150 grams and healthy conditions characterized by no anatomical abnormality and active movement. By using the Federer formula, 24 rats were used in this study. All rats were first adapted for one week and then anesthetized with ketamine and xylazine intramuscularly. The hair on the dorsal side near the spine was shaved, then the skin of the rat in that area was stretched between the fingers, and then an incision was made using a scalpel with a length of 1–1.5 cm and a depth of 2–3 mm (Gunawan et al. 2021).

Twenty-four rats were divided into four groups consisting of six rats each, i.e., two control groups

(K1 and K2) and two treatment groups (P1 and P2). The control groups were given distilled water topically twice a day, with a treatment duration of three days for K1 and five days for K2. The treatment groups were given cabbage extract topically twice a day, with a treatment duration of three days for P1 and five days for P2. After the treatment for the specified time was complete, the rats were euthanized, and the wounded tissues were obtained and put into a container containing 10% formalin for fixation and then processed into histological preparations with hematoxylin and eosin (HE) staining. Histological observations were made by counting the number of macrophages and blood vessels in the wound tissue using a light microscope with 400x magnification that was carried out in three fields of view (Sadikim et al. 2018). The collected data was then analyzed with the Shapiro-Wilk normality test, Levene homogeneity test, and independent sample t-test with $p<0.05$. The statistical analysis in this study used IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, N.Y., USA).

RESULTS

The healing parameters observed in this study were the number of macrophage cells and newly formed blood vessels. The descriptive analysis results of the macrophage cells are presented in Table 1. The descriptive analysis results of the blood vessels are presented in Table 2.

Table 1. Distribution of the rats' macrophage cell count.

Group	Mean	Standard deviation	Minimum	Maximum
K1	30.00	2.828	26	33
K2	46.50	2.168	44	50
P1	35.33	2.733	33	40
P2	42.50	2.811	38	46

Table 2. Distribution of the rats' blood vessel count.

Group	Mean	Standard deviation	Minimum	Maximum
K1	13.67	2.503	10	17
K2	31.33	3.933	25	35
P1	20.67	3.204	17	25
P2	25.17	3.125	20	28

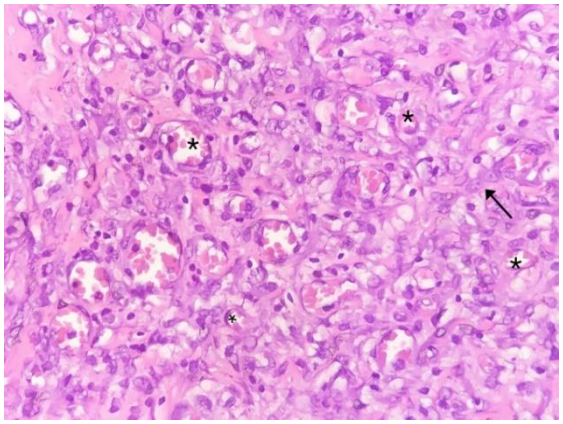
The Shapiro-Wilk normality test was performed and showed that the data on the macrophage and blood vessel counts in all groups (K1, K2, P1, and P2) were normally distributed ($p>0.05$). Afterwards, a homogeneity test was carried out using the Levene test, which showed that the data on the macrophage and blood vessel counts were homogeneous. There was no difference in data variation among all groups ($p>0.05$). The results of the normality and homogeneity tests revealed that the average numbers of macrophages and blood vessels were normally distributed and homogeneous. Therefore, an independent sample t-test was performed to determine the difference in the numbers of macrophages and blood vessels in skin wound tissue samples between the rats that received cabbage extract and those that received placebo (distilled water). The results of the test on macrophage count are presented in Table 3, while the results of the test on blood vessel count are shown in Table 4.

Table 3. Independent sample t-test of macrophage cells.

Groups	p
Day 3 (K1 and P1)	0.008
Day 5 (K2 and P2)	0.020
K (control group day 3 and day 5)	0.000
P (treatment group day 3 and day 5)	0.001

Table 4. Independent sample t-test of blood vessels.

Groups	p
Day 3 (K1 and P1)	0.002
Day 5 (K2 and P2)	0.013
K (control group day 3 and day 5)	0.000
P (treatment group day 3 and day 5)	0.034



Notes: (*) shows blood vessels; (→) shows macrophage cells.

Figure 1. Microscopic picture of rat skin wound with topical administration of cabbage extract on day 5.

DISCUSSION

In this study, the average number of macrophages

was significantly higher on the third day and lower on the fifth day after the provision of cabbage extract. Immediately after injury, the danger signals, which are damage-associated molecular patterns (DAMP) released by damaged cells or pathogen-associated molecular patterns (PAMP) released by invading pathogens, will be detected by resident macrophages and will promote the activation of proinflammatory macrophages (M1) to cooperate with neutrophils in the phagocytosis of microbes, dead cells, cellular debris, and apoptotic neutrophils to produce proinflammatory mediators, as well as chemokines to recruit additional leukocytes to strengthen inflammation (Landén et al. 2016). The expression of various proinflammatory mediators and chemokines to recruit more leukocytes in the early phase of wound healing is in accordance with the results of the study on the third day, where the macrophage counts found in the control and treatment groups were significantly higher than the macrophage count of the same group on the fifth day. However, the number of macrophages in the treatment group on the third day was significantly higher than the number of macrophages in the control group on the same day. This showed that topical application of cabbage extract could accelerate the wound healing process by promoting faster migration of inflammatory cells to the wound site (Sarandy et al. 2015). The rapid macrophage migration in the treatment group was attributed to the effect of increasing the phagocytic capacity of macrophages by sulforaphane and the deactivation of macrophage migration inhibitory factor (MIF), which is an important inflammatory cytokine (Tilg 2015). In addition, flavonoids can induce or inhibit various enzyme systems in mammals, and some of these enzymes are involved in important pathways in the regulation of cell division and proliferation and inflammatory responses (Panche et al. 2016).

Macrophages are important inflammatory cells in the wound healing process that can change their phenotype from proinflammatory macrophages (M1) early in the process to anti-inflammatory macrophages (M2) in the middle stages of the healing process to continue coordinating wound repair (Shook et al. 2016). After the wound is clean, there will be an activation of anti-inflammatory macrophages (M2) that produce collagen precursors, completing the inflammatory phase due to prolonged M1 activation by downregulating proinflammatory tumor necrosis factor alpha (TNF-α) and interleukin 12 (IL-12), expressing anti-inflammatory mediators such as interleukin-1 receptor (IL-1R) and interleukin 10 (IL-10), promoting fibroblast migration and proliferation, producing vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-β), VEGF, insulin-like growth factor-1 (IGF-1), and high amounts of platelet-derived growth factor (PDGF) to promote angiogenesis (Landén et al. 2016, Krzyszczyk et al. 2018, Cañedo-Dorantes & Cañedo-Ayala 2019).

The decrease in the number of macrophages in the treatment group compared to the control group on the fifth day indicated a change in the phenotype of macrophages from pro-inflammatory to anti-inflammatory due to changes in the microenvironment (Torregrossa et al. 2021). In addition, the decrease in the number of macrophages also showed that on the fifth day, the wound healing process in the treatment group had entered a further proliferative phase compared to the control group on the same day. This was because the number of macrophages started to increase during the inflammatory phase, reached a maximum amount during the proliferative phase, and decreased progressively during the remodeling phase (Cañedo-Dorantes & Cañedo-Ayala 2019). Therefore, it was concluded that the effect of cabbage extract on wound healing met the characteristics of the ideal inflammatory response, which was fast and destructive but specific and not excessive.

The administration of cabbage extract showed a significant increase in the average number of blood vessels on the third day, but a decrease was observed on the fifth day. In the proliferative phase, there is massive migration and proliferation of keratinocytes mediated by macrophages to recover the epidermis and tissue regeneration with fibroblast migration and restoration of blood vessels (Shook et al. 2016). A significant increase in blood vessels in the treatment group on the third day (P1) proved that cabbage extract affects the wound healing process by accelerating angiogenesis for the reason that increased blood vessel formation can shorten wound healing time. In the wound healing process, VEGF expression normally increases in the first two to five days after injury to stimulate fibroblast proliferation and extracellular matrix production (Wang et al. 2016, Kurniawati et al. 2022). The higher number of blood vessels on the third day was in accordance with the number of macrophages on the same day because, apart from being phagocytes, the inflammatory phase macrophages also affect the formation of new blood vessels by expressing VEGF to stimulate angiogenesis as granulation tissue begins to form. This is considered a normal wound-healing mechanism (Torregrossa et al. 2021).

The number of blood vessels was in accordance with the number of macrophages in the treatment group on the same day. On the third day, the higher number of macrophages reaching the maximum number earlier than the control group also

accelerated the proliferation process because the macrophages that regulate the proliferative phase are anti-inflammatory macrophages (M2), which produce proangiogenic factors such as insulin-like growth factor (IGF-1), transforming growth factor-β (TGF-β), and vascular endothelial growth factor (VEGF) (Serra et al. 2017). This was supported by a significant decrease in blood vessel number, which was also in line with a decrease in macrophage number found in the treatment group on the fifth day, indicating that granulation tissue formation has occurred. While in the control group, on the same day, granulation tissue formation still occurred in an earlier or recently started phase because the blood vessel count of the group was still significantly higher.

The faster development of vascularization in the treatment group was also influenced by the strong antioxidant activity of flavonoids, such as phytochemicals with high amounts in cabbage, as they inhibit lipid peroxidation to increase vascularity. The effect of cabbage extract in inhibiting lipid peroxidation was proven in a study by Kim et al. (2020) in which three doses of cabbage extract (25, 50, and 100 mg/kg) were given for seven days and ranitidine hydrochloride was given for one day before the rat stomach was induced with hydrochloric acid (HCl) or ethanol to induce ulcerative lesions. At three doses of extract and ranitidine hydrochloride, there was a significant decrease in malondialdehyde levels. The decrease in malondialdehyde and catalase levels showed that cabbage extract at 100 mg/kg was significantly more potent than ranitidine hydrochloride. The phenolic compounds and glucosinolate present in cabbage also have the effect of reducing oxidative stress through the activation pathway of nuclear factor erythroid 2–related factor 2 (Nfr2), which is a transcription factor that can increase the transcription of various antioxidants and detoxification enzymes, thereby reducing the oxidative damage caused by reactive oxygen species (Sanlier & Guler Saban 2018, Ryou et al. 2021, Uuh-Narvaez & Segura-Campos 2021).

Strength and limitations

This study provides data regarding how cabbage extract can accelerate the wound healing process precisely by promoting the early migration of macrophages and accelerating the rate of angiogenesis. Studies on cabbage as an anti-inflammatory agent have been carried out before. However, microscopic parameter calculations in the form of macrophages and new blood vessels have not been performed. Time and cost limitations caused this study to only observe two microscopic parameters, while there were other parameters that could be observed as well.

CONCLUSION

Cabbage extract can accelerate the inflammatory and proliferative phases of wound healing by accelerating the migration of macrophages in the inflammatory phase so that the angiogenesis process also occurs faster. Cabbage extract also limits the degree of inflammation by significantly reducing the number of macrophages and blood vessels in the proliferative phase which indicated that granulation tissue had formed and the proliferative phase had reached a more advanced stage.

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Conflict of interest

None.

Ethical consideration

This research has been approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, with certification No. 139/EC/KEPK/FKUA/2021 on 4/6/2021.

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Author contribution

NUT carried out the treatment of experimental animals and drafted the manuscript. WS contributed to the histological observations of macrophages and blood vessels. S contributed to the revisions of the manuscript.

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Original Research Report

BACTERIAL PROFILE AND ANTIBIOTIC USE IN CATHETER-ASSOCIATED URINARY TRACT INFECTION PATIENTS AT A TERTIARY REFERRAL HOSPITAL IN SURABAYA, INDONESIA

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ABSTRACT

Catheter-associated urinary tract infection (CAUTI) is the most typical type of infection associated with healthcare. This infection constitutes a significant health problem due to its complications and frequent recurrence. The high annual incidence of CAUTI leads to infectious complications and a high cost of treatment. Antibiotic-resistant bacteria further complicate the problem because multiresistant pathogenic bacteria are often responsible for these infections. Moreover, the lack of a comprehensive case reporting system in Indonesia hinders the determination of CAUTI incidence. This study aimed to determine the bacterial profile and antibiotic use in CAUTI patients (n=22) at the Internal Medicine Inpatient Unit of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. This retrospective descriptive study collected data from patient medical records to describe the incidence, type of bacteria, sensitivity patterns, and antibiotic use in CAUTI patients. The data collection process included collecting medical records, recording information on data collection sheets, and recapitulating and analyzing the data. The criteria for CAUTI diagnosis were a bacterial count of ≥105 CFU/mL and a catheterization that lasted more than 48 hours. Samples were selected using non-probability sampling, also known as saturation sampling. Meanwhile, variables analyzed in this study were the type of pathogenic bacteria, antibiotics used, age, and sex. Descriptive analysis was employed to analyze the data. The findings suggested that the most common bacteria were *Escherichia coli*, with eight of nine bacteria showing extended-spectrum beta-lactamase (ESBL) production. Female patients aged 35–65 years were the majority of the CAUTI patients. Amikacin was the antibiotic with the highest sensitivity (90.9%), while ceftriaxone was the most common type of antibiotic administered to the patients. In addition, intravenous (IV) administration was the most common route of antibiotic administration, with an average therapy duration of seven days. In conclusion, identifying the type of pathogenic bacteria and performing antibiotic susceptibility testing can help minimize antibiotic resistance and prevent more severe complications in CAUTI patients.

Keywords: Catheter-associated urinary tract infection; healthcare-associated infections; *Escherichia coli*; infectious disease

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Highlights:
1. This study examined patients with catheter-associated urinary tract infections (CAUTI) to find the prevalent bacteria and the frequency of antibiotic use.
2. Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* sensitive to amikacin was found to be the most common pathogen in CAUTI, while ceftriaxone was the most commonly used antibiotic.
3. The findings of this study encompass patients profiles, which may help in understanding patient needs and determining more effective CAUTI treatments.

INTRODUCTION

Catheter-associated urinary tract infection (CAUTI) is an infection of the urinary tract brought on by a tube that has been implanted to drain urine from the bladder. Patients having indwelling catheters at the

time of the infection or within 48 hours of it occurring are considered to have CAUTI (Press & Metlay 2013, Chuang & Tambyah 2021). CAUTI is the most common healthcare-associated infection, accounting for approximately 1 million cases per year in the United States. It is the most common cause of secondary bloodstream infections. The risk factors for CAUTI include age, female gender, diabetes mellitus, and a long duration of catheter insertion. The duration of catheter insertion is the most important factor in the development of bacteriuria (Werneburg 2022). In 2011, there were an estimated 93,000 CAUTI cases. It accounted for more than 13,000 deaths from all healthcare-related urinary tract infections in the United States (Letica-Kriegel et al. 2019). The prevalence of CAUTI ranges from 2.7% to 16% in Medan, Indonesia. However, the total incidence of CAUTI in Indonesia is still unclear due to a flawed case-reporting system (Anggi et al. 2019, Sitepu & Putra 2019). This study is required to establish the precise incidence of CAUTI in Indonesia and at local area levels. Therefore, it is anticipated that CAUTI guidelines can be established to prevent complications from this infection and lower the level of antibiotic resistance in Indonesia. The incidence rate is still unknown in Indonesia as there are only a few CAUTI studies in the country, despite the world's and Indonesia's increasing levels of antibiotic resistance.

Gram-negative bacterial species, including *Escherichia coli* and *Enterococcus* spp., are the most common organisms that cause CAUTI. In addition to pathogenic bacteria, CAUTI can be caused by fungi, one of which is *Trichosporon asahii*, which has been recorded in India (Urs et al. 2018, Werneburg 2022). Fungal organisms other than *Trichosporon asahii* and *Candida* spp. are reported to cause CAUTI. Although CAUTI morbidity and mortality rates are relatively low compared to other healthcare-associated infections, the high annual incidence and overuse of antibiotics can lead to many complications and high costs of care (Rishpana 2015, Werneburg 2022).

Increased resistance of pathogenic bacteria to antibiotics is a common problem because multiresistant pathogenic bacteria often cause this infection. Inappropriate use of antibiotics for patients with urinary catheters is a significant factor in the increase in bacterial resistance (Köves et al. 2017). In Pakistan, resistance to ceftazidime and cefotaxime antibiotics against pathogenic bacteria that cause CAUTI occurred. By knowing the pattern of resistance of pathogenic bacteria, it is hoped that if there are CAUTI patients, they can find out the possible type of bacteria causing it and use empirical antibiotic therapy to prevent further resistance and more severe complications (Sayeed et al. 2019). This present study was conducted at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, which is a referral hospital and the largest in eastern Indonesia with diverse patient characteristics. Therefore, we chose the hospital as the research location and expected the research questions to be answered correctly. This study aimed to investigate the bacterial profile and antibiotic use by examining urine specimens taken from patients diagnosed with CAUTI.

MATERIALS AND METHODS

This study was a retrospective descriptive study of 22 hospitalized patients diagnosed with CAUTI. The patients were over the age of 18. Data were obtained sequentially from urine culture, bacterial identification, and antibiotic susceptibility tests carried out at the Clinical Microbiology Laboratory, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, for one year between May 2020 and May 2021. Medical records were used to extract additional information about patient profiles. The data were collected and analyzed retrospectively. IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, N.Y., USA) was used to descriptively evaluate the data (Utami et al. 2022).

Patients with urinary indwelling catheters provided urine samples at the beginning of the study. When the bacterial count was greater than or equivalent to 105 CFU/mL and the catheterization lasted more than 48 hours, the CAUTI diagnosis was determined. Any urine samples that had two or more distinct bacterial colonies were not taken into consideration for further investigation (Utami et al. 2022). This study was ethically approved by the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, with reference No. 0616/LOE/301.4.2/IX/2021 dated 27/9/2021.

RESULTS

Patient characteristics

A total of 18 (81.82%) of the 22 patients were women. Most patients were in the age groups of 36–45 years and 56–65 years. A summary of the patients' age and sex distribution is provided in Table 1.

Bacteriological investigations

Most of the bacteria that caused CAUTI are Gram-negative bacteria (n=10). *Escherichia coli* was the most common bacterium in this study, with a total of nine cases (56%). Eight of nine *Escherichia coli* isolates were extended-spectrum beta-lactamase

(ESBL)-producing strains. The distribution of bacteria is provided in Table 1.

Table 1. Patient demographics, pathogenic bacteria, and antibiotics used in CAUTI.

Category		Total (n, %)
Sex	Male	4 (18.18%)
	Female	18 (81.82%)
Age	36–45 years	7 (31.82%)
	46–55 years	5 (22.73%)
	56–65 years	7 (31.82%)
	>65 years	3 (13.63%)
Bacteria	*Escherichia coli*	9 (56%)
	Staphylococcus haemolyticus	1 (6%)
	Moraxella species	1 (6%)
	Enterococcus faecalis	2 (13%)
	Candida species	3 (19%)
Administration route	Oral	(21.05%)
	Intravenous	(78.95%)
Treatment duration	Seven days	21 (95.45%)
	Three days	1 (4.55%)
Antibiotics	Ceftriaxone	17 (45.94%)
	Moxifloxacin	4 (10.81%)
	Cefixime	4 (10.81%)
	Cefoperazone-sulbactam	3 (8.1%)
	Ciprofloxacin	3 (8.1%)
	Meropenem	2 (5.4%)
	Ampicillin-sulbactam	1 (2.7%)
	Cotrimoxazole	1 (2.7%)
	Cefotaxime	1 (2.7%)
	Levofloxacin	1 (2.7%)

Patterns of antibiotic use

The most common route of antibiotic administration in CAUTI patients was intravenous (78.95%). The average duration of antibiotic therapy in CAUTI patients was seven days (n=21, 95.45%). The most common antibiotic for CAUTI patients was ceftriaxone (n=17). Table 1 presents the complete data on patterns of antibiotic use.

Antibiotics susceptibility

Antibiotics that had a high susceptibility to *Escherichia coli* were amikacin (90.9%), meropenem (86.4%), imipenem (77.3%), piperacillin-tazobactam (63.6%), and cephalexin (59.1%). However, several antibiotics showed lower susceptibility to *Escherichia coli* or even no susceptibility at all, i.e., cefazolin and ciprofloxacin. Table 2 shows the complete data on antibiotic susceptibility.

Table 2. The susceptibility rate of antibiotics to *Escherichia coli*.

Antibiotics	*Escherichia coli*
Amikacin	90.9 %
Meropenem	86.4 %
Imipenem	77.3 %
Piperacillin-tazobactam	63.6 %
Cephalexin	59.1 %
Cefoperazone-sulbactam	36.4 %
Tigecycline	27.3 %
Amoxicillin-clavulanic acid	22.7 %
Cefepime	22.7 %
Fosfomycin	22.7 %
Trimethoprim-sulfamethoxazole	22.7 %
Ceftazidime	18.2 %
Ceftriaxone	18.2 %
Gentamicin	18.2 %
Cefotaxime	13.6 %
Ampicillin-sulbactam	13.6 %
Aztreonam	13.6 %
Moxifloxacin	13.6 %
Tetracycline	13.6 %
Piperacillin	9.1 %
Chloramphenicol	4.5 %
Levofloxacin	4.5 %
Cefazolin	0
Ciprofloxacin	0

DISCUSSION

Patient characteristics

The shorter urethra in women than in men makes the female urinary tract more vulnerable, increasing the incidence of CAUTI. The current findings indicated that CAUTI was more prevalent in women. These findings are corroborated by another study, which stated that 59% of their patients with CAUTI were women (Smith et al. 2019). The incidence of CAUTI is determined by age and gender. In this study, patients in the age groups of 36–45 years and 56–65 years were the largest populations who experienced CAUTI, or about 31.82% of the entire population. This is consistent with an earlier study by Hariati et al. (2019) who found that CAUTI was more prevalent in people over 60 years old. Older age and immobility were two additional significant risk variables, although they could be influenced by other factors (Setyorini et al. 2019). Patients with diabetes mellitus have a significant risk of developing urinary tract infections since their urine is a source of microbial growth, and they typically have immunocompromised states. As a result, they require careful blood glucose management to stop the development and spread of CAUTI (Li et al. 2019).

Bacteriological investigations

The majority of bacteria that cause CAUTI come from the perineum's endogenous microbiota, which ascends the urethra to the bladder along the catheter's outer surface. A smaller percentage of microorganisms (34%) were added by intraluminal contamination of the collecting system from exogenous sources, typically as a result of organisms being transferred from the hands of healthcare workers. In a study at a hospital, patient-to-patient transmission accounted for 15% of instances of bacteriuria connected with health care (Clarke 2014).

The typical test for identifying the bacteria responsible for CAUTI is a urine culture. There are numerous methods for collecting urine cultures, including the straight catheter technique, the mid-stream technique with or without purging, and suprapubic aspiration. In pediatric patients, the technique of collecting diapers and sterile bags is the most common collection technique. The best way to prevent microorganisms from contaminating a specimen is through suprapubic collection, especially in the distal urethra. This approach is rarely used since it is invasive, uncomfortable for patients, lacks an indication, and wastes resources. The next best method is to collect urine using just one catheter (straight catheter approach) (Karah et al. 2020). However, this treatment is only applied when necessary because it is labor-intensive and has the risk of introducing germs into the bladder, which can lead to a UTI. In a comparison between suprapubic aspiration and the single catheter method, the colony counts from the obtained samples match rather well. Whereas in adult patients, the mid-stream technique is the most commonly used because it is non-invasive (Sinawe & Casadesus 2023).

The patient's history, clinical signs and symptoms, and other laboratory tests should all be taken into consideration when interpreting the results of a urine culture. Nonetheless, many patients are diagnosed with CAUTI with negative urine cultures because the clinical signs and symptoms suggest a urinary tract infection. Negative urine culture results in patients with CAUTI are influenced by the number of days of urinary catheter insertion and the patient's maximum temperature (Podkovik et al. 2019, Karah et al. 2020).

Patterns of antibiotic use

Treatment with antibiotics for CAUTI aims to eliminate bacteria and bacterial colonies, relieve symptoms, treat sepsis, and prevent sequelae. Musinguzi et al. (2019) stated that the pathogenic bacteria of CAUTI showed resistance to commonly prescribed antibiotics. *Escherichia coli* has the highest antibiotic resistance to all antibiotics. All Gram-negative bacteria were sensitive to imipenem (100%) and gentamicin (83%), but the high cost of these antibiotics and the nephrotoxic effect of gentamicin continue to limit their use (Musinguzi et al. 2019).

The current research suggests that CAUTI is the only case where systemic antibiotic treatment is necessary. When a CAUTI is identified, a new catheter should be used, and treatment should begin initially with broad-spectrum antibiotics by considering the local susceptibility patterns and later modifying the treatment according to the results of urine or blood culture tests (Karah et al. 2020). Analysis of prognostic factors for CAUTI caused by bacteremia catheters revealed a poor prognosis and higher mortality linked to incorrect empirical treatment. The likelihood that the bacteremia was brought on by antibiotic-resistant uropathogens was much higher, and the likelihood that such pathogens had previously been exposed to antibiotic therapy and associated with bacteremia in a medical setting was a predictive factor (Tenke et al. 2014).

The most typical method of administering antibiotics to CAUTI patients is intravenous, particularly for those with significant systemic symptoms such as high fever, hemodynamic instability, poor oral tolerability, and reduced absorption. At 48 to 72 hours, the clinical response can be assessed. Parenteral antibiotic therapy can be reviewed after culture results are available. For many patients, oral therapy can also be selected after reviewing the results of the urine culture or after 72 hours of intravenous antibiotics (Spoorenberg et al. 2014).

If there is an indication, antibiotics should be chosen according to the urine culture results. It is also advised to initiate empirical antibiotic therapy while awaiting the findings of the culture if the patient exhibits moderate to severe symptoms. Ampicillin and gentamicin are still suitable as initial empiric therapy alternatives for parenteral antibiotic therapy. Broad-spectrum beta-lactam antibiotics (such as cefotaxime, ceftriaxone, and piperacillin or tazobactam) and other cell wall-active drugs are an option for empirical parenteral therapy. Antifungal medications should be taken for 14 days to treat symptomatic CAUTI brought on by *Candida* spp. (Clarke 2014).

The majority of CAUTI patients receive antibiotic treatment for seven days. Furthermore, according to a study by Clarke Clarke (2014) seven days of antibiotic therapy can be sufficient in cases when there is a rapid clinical worsening, such as a drop in temperature within 72 hours. For those who have a

delayed reaction, a small amount of antibiotics is administered for 10–14 days. The most common type of antibiotic given to CAUTI patients is ceftriaxone. This medication is often administered to patients with CAUTI caused by *Enterococcus* spp., elderly patients, and those who experience urinary tract infections due to catheterization. These results are supported by previous study by Karlović et al. (2018).

Antibiotic susceptibility

Some antibiotics are resistant to both Gram-negative and positive bacteria. The rise of ESBL uropathogenic strains can result in the development of beta-lactam antibiotic resistance, particularly for Gram-negative bacteria. In this study, ESBL-producing *Escherichia coli* were resistant to ciprofloxacin and cefazolin. This resistance occurred because ESBL is an enzyme capable of hydrolyzing the beta-lactam ring, which caused a loss of beta-lactam antibiotic activity (Bush 2018).

Escherichia coli has a varying sensitivity to several antibiotics. In this study, the sensitivity levels of *Escherichia coli* to amikacin (90.9%), meropenem (86.4%), imipenem (77.3%), piperacillin-tazobactam (63.6%), and cephalexin (59.1%) were found. However, some antibiotics were not sensitive to *Escherichia coli*, i.e., cefazolin and ciprofloxacin, with a sensitivity level of 0%. These results are consistent with previous studies, which revealed that *Escherichia coli* was sensitive to amikacin (82.6%), piperacillin-tazobactam (78.2%), nitrofurantoin (82.1%), and imipenem (98.9%) (Niranjan & Malini 2014). The sensivity of *Escherichia coli* to ampicillin, cefuroxime, ceftriaxone, norfloxacin, and ciprofloxacin varied between 11% and 25%. In another study, *Escherichia coli* was sensitive to ampicillin (39.1%), amoxicillin-clavulanic acid (64.9%), cefotaxime (94.9%), gentamicin (93.9%), and amikacin (98.6%) (Daoud et al. 2020).

Strength and limitations

The limitation of this study is that it is a retrospective study using patient medical records carried out in one center, so a small number of samples were obtained. The data only represent that specific center and may not be representative or cannot be used in other centers or hospitals. The impact of this research on clinical practice is that it can be used as a reference for intervention or management of CAUTI patients at the center or in the local area. It is expected to help reduce the level of antibiotic resistance, which has been increasing recently. For further research, prospective research can be carried out in several areas or centers so that a large number of samples can be obtained and comparisons can be made with other centers to find out the shift in pathogenic bacteria.

CONCLUSION

Escherichia coli was the most common bacterial pathogen associated with CAUTI, with the majority of cases attributed to gram-negative bacteria. Sensitivity testing showed that amikacin was effective against *Escherichia coli*, while cefazolin and ciprofloxacin were resistant to *Escherichia coli*. Intravenous administration was the preferred route for antibiotic administration, with an average therapy duration of seven days, and the most frequently prescribed antibiotic was ceftriaxone. Further research is recommended to be conducted in at least two different hospitals to represent a larger population and to compare the causative bacteria. In addition, the research can be carried out periodically to investigate any shifts in the pattern of pathogenic bacteria.

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Conflict of interest

None.

Ethical consideration

This study was conducted with the approval from the Health Research Ethics Committee, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, with reference No. 0616/LOE/301.4.2/IX/2021 dated 27/9/2021.

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Author contribution

EAV contributed to the conceptualization and design as well as the data collection, analysis, and interpretation. TPA participated in the drafting, design, critical revision, and final approval of the article for publication. ADWW made crucial revisions to the article and approved the final draft before it was published. BER made crucial revisions to the article and approved the final draft before it was published.

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Original Research Report

EFFECTS OF *Moringa oleifera* LEAF EXTRACT ON THE LIVER OF EXPERIMENTALLY-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

Diabetes mellitus is known as a risk factor for nonalcoholic fatty liver disease (NAFLD) which can progress to nonalcoholic steatohepatitis (NASH) and eventually lead to hepatocellular carcinoma (HCC) through various stages, including necro-inflammatory fibrosis, cirrhosis, and hepatitis. *M. oleifera* leaves contain flavonoid antioxidants, which inhibit reactive oxygen species (ROS) and oxidative stress in diabetes mellitus. This study aimed to investigate the potential of *M. oleifera* leaf extract at a dosage of 1,000 mg/kgbw to inhibit liver tissue fibrosis in diabetic rats. This study used a true experimental method with a post-test-only control group design. This study was conducted at the Faculty of Medicine, Universitas Jember, Jember, Indonesia, from November 2021 to January 2022 on 27 male Wistar rats that were divided into three groups of nine rats. The rats were induced with streptozotocin and *M. oleifera* leaf extract at a dosage of 1,000 mg/kgbw. Masson's trichrome staining and the Meta-analysis of Histological Data in Viral Hepatitis (METAVIR) scoring system were used to measure liver tissue fibrosis. Data were analyzed using the Kruskal-Wallis and Mann-Whitney tests to examine significant differences between groups. The results showed a significant difference in the degree of liver tissue fibrosis between the control and diabetes groups (p=0.00) as well as the diabetes and treatment groups (p=0.003). However, the results did not show any significant differences between the control and treatment groups (p=0.270). These findings suggested that administering *M. oleifera* leaf extract at a dosage of 1,000 mg/kgbw can inhibit liver tissue fibrosis. In conclusion, this study provides evidence that administering *M. oleifera* leaf extract can inhibit liver tissue fibrosis in diabetic rats.

Keywords: Diabetes mellitus; *M. oleifera* leaves; liver fibrosis; nonalcoholic fatty liver disease (NAFLD)

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Highlights:
1. This study examined the antioxidant flavonoid compounds derived from naturally sourced *Moringa oleifera* leaves.
2. *Moringa oleifera* leaf extract was able to prevent tissue fibrosis and liver cirrhosis in diabetic rat models through the nonalcoholic fatty liver disease (NAFLD) pathway.

INTRODUCTION

Diabetes Mellitus denotes a hyperglycemic condition due to a lack of insulin, insulin resistance, or both (Hardianto 2021). The World Health Organization (2020) reports that diabetes mellitus is the sixth largest cause of death worldwide. It is reported that approximately 1.3 million people die before reaching age 70 years old due to diabetes mellitus, while its prevalence is around 150 million people worldwide (Nasution et al. 2021). Indonesia is reported to be a country with the sixth-highest population with diabetes mellitus, with approximately 10.3 million people aged 20 to 79 years old (Minister of Health of the Republic of Indonesia 2018).

Diabetes mellitus is a risk factor for nonalcoholic fatty liver disease (NAFLD), which triggers nonalcoholic steatohepatitis (NASH) through necroinflammatory fibrosis, and cirrhosis, and eventually results in hepatocellular carcinoma (HCC) (Bellentani 2017). NAFLD prevalence data showed that more than 70% of type 2 diabetes

mellitus patients have NAFLD, and 20% of these NAFLD developed liver fibrosis and ended up becoming HCC (Mitra et al. 2020). This is due to a lack of insulin secretion which can trigger lipolysis in adipose cells and resulting in an increase of the amount of free fatty acids in the blood that go to the liver and muscles (Leite 2014). In diabetes mellitus patients, NAFLD can also activates Kupffer cells in the liver and release hepatic inflammatory mediators (IL-1β, TNFα, IL-6) to indicate the occurrence of inflammation and fibrosis in the liver (Xia et al. 2019).

When diabetes mellitus occurs, the liver will find difficulties to export triglycerides in the form of Very Low-Density Lipoprotein (VLDL). As a result, there will be an accumulation of fat in the liver which encourages fatty liver and inflammation of hepatocytes due to free radicals resulting from the oxidation of fatty acids by mitochondria and lysosomes (Hazlehurst et al. 2016). Diabetes mellitus may cause oxidative stress through the increased production of reactive oxygen species (ROS). The continuous release of ROS might cause an imbalance between prooxidant and antioxidant, stimulating proinflammatory mediators. Increased ROS leads to excessive oxidative stress and eventually results in NAFLD, NASH, fibrosis, and HCC (Safithri 2018). Addressing this condition, external antioxidant compounds like flavonoids, vitamin C, E, and pro-vitamin A are required to balance or inhibit ROS (Tukiran et al. 2020).

Previous study showed that flavonoid content (i.e., quercetin) in *M. oleifera* leaves can potentially serve as hepatoprotectives, hypocholesterolemia, hypo-lipidemia, and anti atheroschlerotics with anti-inflammatory and antioxidant effects (Lin et al. 2018). Flavonoid was also reported to inhibit ROS release, thus reducing the severity of NAFLD in diabetes mellitus (Akhlaghi 2016). The result of this study is expected to provide a reference for further study on the benefits of *M. oleifera* leaves in preventing fibrosis in patients with diabetes mellitus.

MATERIALS AND METHODS

This study was categorized as a true experimental study with a post-test-only control group design. The experimental unit in this study was male Wistar rats (*Rattus norvegicus*) induced with streptozotocin (STZ) and received a 1,000 mg/kgbw *M. oleifera* leaf extract. The *M. oleifera* leaf extract was obtained from the pharmacology laboratory of Faculty of Medicine, University Jember, Jember, Indonesia, and the leaf extract was made using maceration technique with 96% ethanol solvent. The use of 96% ethanol can produce the highest number of flavonoids in the leaf extract. The manufacturing process began with 5 kg of leaves washed clean with running water and then dried in an oven at a temperature of 60°C. The leaves, which have been dried, were then smoothed using a blender to obtain 600 grams of fine *M. oleifera* leaves powder. Then, the powder was filtered to obtain the exact smooth level. The subsequently thin powdered leaves were processed in a glass cup with 6,000 mL of 96% ethanol under tightly closed conditions and not exposed to sunlight. A 72-hour maceration was performed, with stirring every 24 hours. The processed leaves were then filtered using Whatman filter paper, and then they were evaporated to obtain thick extract preparation using a water bath at a temperature of 70°C (Nortjie et al. 2022). The extract was then dissolved using sodium carboxymethyl cellulose to obtain extract suspension that would be given to the rats. The liver histopathology preparation was made in the biomedical laboratory of Faculty of Dentistry, University of Jember. The histopathological preparations began with the process of fixation, dehydration, and impregnation, continued with deparaffination, and finally the staining using Masson's trichrome was carried out. The use of Masson's trichrome coloring has become a preferred method for identifying and detecting histopathological morphological changes from fibrosis in patient biopsies and animal models of fibrose (van de Vlekkert et al. 2020). The liver fibrosis reading was performed by a medical specialist in Anatomic Pathology Laboratory of Dr. Soebandi Regional Hospital, Jember, Indonesia. The reading of the preparation was done at one field of view using a light microscope with a 100x magnification. The results of the observations were then viewed and interpreted based on the criteria of the Meta-analysis of Histological Data in Viral Hepatitis (METAVIR) scoring system. This study was conducted for three months, from November 2021 to January 2022.

Wistar rats obtained in this study were divided into three treatment groups, each consisting of nine rats. The rats in the normal control group were given normal saline intraperitoneally followed by per-oral normal saline once per day for four weeks. Meanwhile, those in the diabetic control group were induced by 45 mg/kgbw of STZ, which was dissolved with citrate buffer once via intraperitoneally and followed by per-oral normal saline once per day for four weeks. The treatment group received 45 mg/kgbw intraperitoneal and 1,000 mg/kgbw per-oral once a day for four weeks. After 28 days of treatment, the rats were euthanized using sodium pentobarbital in a dose of 50 mg/kgbw intraperitoneally (American Veterinary Medical Association 2020).

The inclusion criteria for the experimental unit were 2-3 months old male Wistar rats (*Rattus norvegicus*), weighed 200-300 grams and had a fasting blood sugar level of ≥126-400 mg/dL three days after being induced by streptozotocin (Gheibi et al. 2017). The drop-out criterion was the dead rats after being induced with STZ during the experiment. Liver fibrosis was assessed using the METAVIR scoring system, as it is believed to be more sensitive to fibrotic activities (Chengxi et al. 2018). The METAVIR scoring system is semi-quantitative in evaluating fibrosis grade (Choo et al. 2022). The fibrosis assessment results were analyzed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA). Kruskal-Wallis test with significant results of $p<0.05$ was followed up with post-hoc Mann-Whitney test.

RESULTS

The blood sugar test of fasting rats was performed on the third day after being induced using STZ and the rats became diabetic (Saputra 2018). The rats were first fasted for six to eight hours before their blood was taken (Furman 2021). The blood of rats in the diabetic control and treatment groups was taken through the lateral veins of the tail using a glucometer. After the STZ-induced developed hyperglycemia with an average fasting blood sugar level of 405 mg/dL, the following normal saline induction produced a normal fasting average blood glucose level of 105 mg/dL as the normal fasting blood glucose is <126 mg/dL.

Table 1. The METAVIR scoring results.

Groups	No. of samples	Experiments	Liver fibrosis scoring median (IQR)
Normal control	9	Normal	1 (0)
Diabetic control	9	STZ and normal saline	2 (1)
Treatment	9	STZ and 1,000 mg/kgbw of *M. oleifera* leaf extract	1 (1)

Male Wistar rats in this study were divided into three groups, normal control group, diabetic control group, and treatment group. Liver fibrosis was assessed using the METAVIR scoring system. This system currently serves as the most acceptable system for liver fibrosis and necroinflammation assessment. Staging fibrosis using the METAVIR score to assign a score ranging from F0 (no fibrosis) to F4 (cirrhosis) is the gold standard in liver biopsy (Chengxi et al. 2018). The METAVIR scoring results of rats' liver fibrosis in normal control group, diabetic control group, and treatment group are presented in Table 1.

Table 1 displays the average result of liver fibrosis using the METAVIR scoring system. The highest liver fibrosis mean score was found in diabetic control group, followed by the treatment group, and the normal control group. The highest average (2.22) was found in the diabetic group, whereas the lowest average (1.11) was found in the normal control group.

Table 2. Kruskall Wallis test result.

	Stages
Kruskal-Wallis H	15.658
Degrees of freedom (df)	2
Asymptotic significance	0.000
Notes: p=0.000	

The Kruskal-Wallis test result, as shown in Table 2, showed a significance value of p=0.000, indicating a significant difference among the three groups. The analysis was continued using post-hoc Mann-Whitney test to compare groups showing significant results. The result of post hoc Mann-Whitney test is presented in Figure 1.

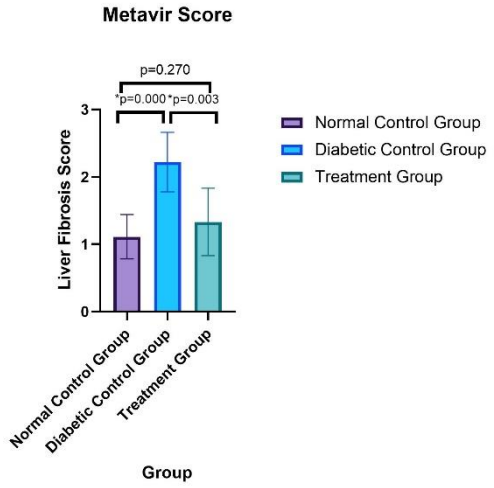

Figure 1. Post-hoc Mann-Whitney test result. *p<0.05.

The post-hoc Mann-Whitney test result ($p<0.05$) indicates a significant difference in rat liver fibrosis assessment among study groups. The normal control group showed a significant fibrosis development compared to the diabetic control group (0.000), but not significant compared to the treatment group

(0.270).

Figure 2 displays the histopathology of rats' liver fibrosis in normal control (A), diabetic control (B), and treatment (C) groups.

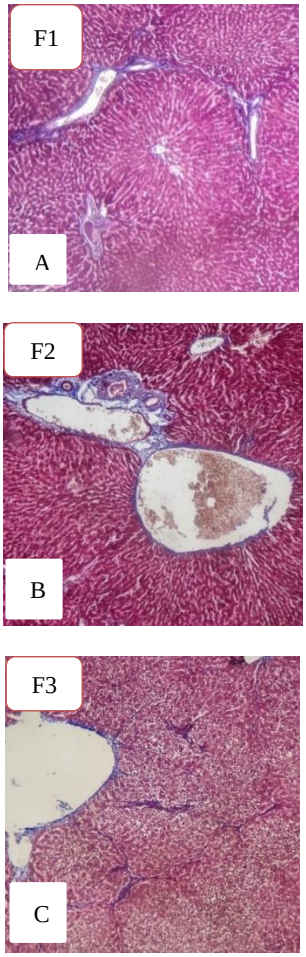

Figure 2. Histopathological liver by Masson's trichrome staining at 100X microscopic magnification of rats' liver fibrosis. (A) Liver fibrosis stage F1 with portal area expansion (normal control); (B) F2 fibrosis with expansion of portal area and marked bridging (treatment group); (C) F3 fibrosis with expansion of portal area and marked bridging, nodules were also noticed (diabetic control).

The result indicates that the administration of 1,000 mg/kgbw *M. oleifera* leaf extract could inhibit liver fibrosis. Figure 2 displays the liver fibrosis observation using the METAVIR scoring system. The normal control group showed a fibrosis expansion in the portal area and collagen deposition, while the diabetic control group showed a fibrosis expansion in the portal area, marked bridging, and a nodule surrounded by collagen deposition. The treatment group exhibited fibrosis with expansion in the portal area, marked bridging and clearly visible collagen deposition. This group also exhibited better results than the diabetic control group.

DISCUSSION

The average score of rats in diabetic control group was found to be higher than in normal control group. The observation showed that diabetic rats exhibited stages F2 and F3 of fibrosis. Similar condition was also reported in the study by Aseer et al. (2015), that 14 days after being induced by STZ, the rats showed damaged liver histopathology associated with fibrosis. This fibrosis was caused by the increase in inflammatory mediators such as TNF-α, TGF-β1, and IL-6, which leads to increased ECM and collagen. In another study, liver steatosis and fibrosis began with the increase in free fatty acid due to insulin resistance induced by STZ (Ramadan et al. 2022).

Diabetes mellitus could increase ROS production. The imbalance between production and elimination of ROS can cause oxidative stress that change the structure and functions of protein, nucleic acid, and even damage the DNA (Moreli et al. 2014). Excessive ROS may induce the increased concentration of proinflammatory mediators such as TNF, IL-6, IL-1β, IL-13, TGF- β1, and Galectin-3 (Fulton et al. 2019). Liver fibrosis process is initiated by HSC activation. Various factors like inflammation and cytokine, especially TGF-β1, may prevent HSCs from maintaining balance between ECM production and degradation. An excessive ECM production is known to serve as pathogenesis of organ fibrosis (Heydarpour et al. 2020, Kim et al. 2020).

In this study, rats in normal control group exhibited the lowest liver fibrosis score. In normal group, all rats exhibited stage-1 liver fibrosis, while it should not have occurred. Adeyemi et al. (2014) stated that administering normal saline to rats with normal fasting blood glucose level for four weeks did not result in liver fibrosis, as identified using HE staining method. Another study by Salih et al. (2014) added that rats in control group with normal saline did not exhibit histological or anatomical differences from those induced by STZ for two, four, and six weeks.

Fibrosis in normal control group may be accounted for several reasons. In this study, liver fibrosis histopathology was identified using Masson's trichrome staining, a staining method sensitive to collagen as the fibrosis marker. Thus, when compared to HE staining, fibrosis may be unidentified. Furthermore, seemingly healthy rats possibly have liver disorders. In this regard, Yang et

al. (2019) reported that during rats' embryogenesis process, mesothelial cells (MCs) derived from septum transversum mesenchyme (STM) induce hepatic stellate cells and mesenchymal perivascular cells. Thus, rats in the normal group could potentially experience cell differentiation from hepatic stellate cells and mesenchymal perivascular to myofibroblast that turn into fibrosis over time. Another study from Zhao et al. (2016) added that MCs form hepatic stellate cells and myofibroblas through epithelial mesenchymal transition (EMT). EMT refers to a process in which epithelial cells lose their polarity and obtain migration capacity during embryogenesis, tissue recovery, organ fibrosis, and tumor metastasis.

Strength and limitations

This study is the initial and first study that discusses the benefits of *Moringa oleifera* extract against liver fibrosis in model diabetic rats. However, this study cannot be directly implemented in humans, and further studies on the effective dosage in human beings are needed.

CONCLUSION

The treatment group receiving *M. oleifera* leaf extract did not exhibit significant results compared to the normal control group but showed significant results when compared to the diabetic control group. Thus, it could be concluded that administering *M. oleifera* leaf extract can inhibit liver tissue fibrosis in diabetic rats. Further research can measure malondialdehyde (MDA) as an indicator of oxidative stress, which is the mechanism of hepatic fibrosis occurrence.

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Conflict of interest

None.

Ethical consideration

This experimental study was approved by the Ethics Committee of the Faculty of Medicine of Universitas Jember with a reference number 1559/H25.1.11/KE/2022 on 21/10/2021.

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None.

Author contribution

WI performed the experiments and drafted the manuscript. SS provided idea and expert opinion. AP performed the data analysis.

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Original Research Report

IMPACT ON THE KIDNEY OF PANCREAS DAMAGE DUE TO STREPTOZOTOCIN-INDUCED HYPERGLYCEMIA

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ABSTRACT

The kidneys are one of the organs affected by microvascular complications due to diabetes mellitus. Hyperglycemia plays an important role in glomerular, mesangial cell, and tubular damage in the kidneys. Metabolic dysregulation, including hyperglycemia, initiates cellular damage in the kidneys. Streptozotocin (STZ) is a chemical compound that is known to damage pancreatic cells and cause hyperglycemia. This study aimed to examine the effects of hyperglycemia on the morphology of the kidneys. Kidney tissues were observed histologically using a light microscope. Samples were taken from the kidneys of experimental animals administered with STZ to induce hyperglycemia. Observation was performed afterwards to investigate any damage to pancreatic cells. A total of 12 kidney samples were divided into two groups: the control group and the STZ-induced group. The samples were prepared before staining with hematoxylin-eosin and Masson's trichrome. The endothelium, podocytes, mesangial cells, and basement membrane of the glomerulus were examined. The tubules of the kidneys were also examined, and the presence or absence of connective tissue formation in both groups was statistically tested. The results suggested a significant difference in tubular damage ($p<0.05$) and an insignificant difference in an increase in the damage of other components of the kidneys ($p>0.05$) in the STZ-induced group. Significant morphological changes were observed in the hyperglycemic renal tubules due to the administration of STZ. In conclusion, STZ-induced hyperglycemia caused damage to the kidney components but overall had no significant impact on the kidney.

Keywords: Diabetes mellitus; glomerulus; streptozotocin (STZ); tubular damage

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Highlights:
1. This study observed the histology of pancreatic β-cell damage without any intervention to the kidneys of the animal models.
2. The histological analysis of the kidneys shows that STZ-induced animal models can be used for assessing kidney abnormalities due to hyperglycemia.
3. A scoring system for the histological analysis was developed to evaluate the changes in the kidney cells.

INTRODUCTION

Streptozotocin (STZ) is a glucose molecule derived from the *Streptomyces griseus* soil bacterium. The bacteria can be used for a broad-spectrum antibiotic that is toxic to pancreatic β-cells (Sundaram et al. 2019). STZ has been shown to selectively harm pancreatic islet cells, resulting in hyperglycemia. The chemical compounds in STZ can be used to investigate tissue damage caused by diabetes. This is important for developing experimental animal models, such as rats, mice, and monkeys. In recent

years, STZ has become the drug of choice for inducing diabetes, especially in rodents, due to its complications (Furman 2015, Giralt-López et al. 2020). The administration of STZ in 50–65 mg/kg doses can cause hyperglycemia without severe ketosis.

The degenerative nature of diabetes mellitus complications results in high mortality and morbidity rates. The World Health Organization reported an increase in the prevalence of type 2 diabetes mellitus globally. Furthermore, the International Diabetes Federation reported that there was an increase in the number of people with type 2 diabetes mellitus between 2013 and 2017. By the year 2045, it was estimated that the number would increase to 693 million (Cho et al. 2018). In the 2018 Basic Health Research report by the Indonesian Ministry of Health, there was an increase of 8.5% in the prevalence of type 2 diabetes mellitus. As the prevalence of diabetes mellitus increased, the probability of complications, such as microvascular, macrovascular, and nervous system disorders, also increased (Soelistijo et al. 2021).

One of the organs affected by microvascular complications caused by diabetes is the kidney. Chronic kidney disease is a potential impact of diabetes mellitus. Moreover, diabetes is the single most common cause of end-stage chronic kidney disease. Approximately 20–40% of diabetes mellitus patients will develop diabetic nephropathy, which is the cause of end-stage chronic kidney disease (Ameh et al. 2019). Hyperglycemia is an important factor in the development of glomerular, mesangial, and tubular damage in the kidney. Kidney cellular damage is initiated by metabolic dysregulation, including hyperglycemia, hyperlipidemia, and insulin resistance. Increased reactive oxygen species due to mitochondrial dysfunction in diabetes is a primary event in the development of complications (Reidy et al. 2014). The role of mesangial cells is to maintain the structure of the glomerular capillaries and regulate glomerular filtration through smooth muscle activity. Hyperglycemia boosts the proliferation and hypertrophy of mesangial cells through increased reactive oxygen species in the cells. This can lead to an increase in matrix production as well as thickening of the basement membrane. Hyperglycemia can also cause an increased expression of vascular endothelial growth factor, which results in increased vascular permeability (Khan et al. 2020). Damage to the glomerulus due to prolonged hyperglycemia can lead to decreased kidney function. Large molecules, such as protein and glucose, can pass through the filtration process if the function of glomerular filtration is compromised. In addition, the function of fluid reabsorption in the renal tubules is also impaired if hyperglycemia occurs.

Long-term hyperglycemia can induce hypoxia, resulting in scar tissue as a response. Fibroblasts in the renal interstitium will differentiate into fibrocytes and fill most of the kidney tissue. Hyperglycemia, in conjunction with transforming growth factor beta (TGF-β), angiotensin (AngII), and advanced glycation end products (AGEs), will induce epithelial-mesenchymal transition (EMT). Additionally, it increases alpha smooth muscle actin (α-SMA) and vimentin expressions, downregulates E-cadherin, damages the epithelial layer, and alters the phenotype of mesenchymal cells, resulting in the formation of scar tissue (Braga et al. 2022). These conditions will clearly worsen kidney function. In the late stages, the renal interstitial space will be filled with scar tissue. A recent study showed that intermittent or chronic hyperglycemia plays an important role in the initiation and persistence of diabetes mellitus complications, including kidney disease (Amorim et al. 2019). This study aimed to determine whether pancreatic beta cell damage that causes hyperglycemia has a direct impact on the kidney. A histological assessment was conducted to observe the presence of the impact.

MATERIALS AND METHODS

This study was part of a larger study by Jusuf et al. (2021) on the effectiveness of manual acupuncture at the EX-B3 Weiwanxianshu point. The study observed the histological images of the pancreas and blood sugar levels of rats with type 2 diabetes. In accordance with the ethical guidelines for the use of animal models in medical research, this study used kidney samples collected from experimental male Sprague-Dawley rats aged 8–10 weeks. The rats were divided into two groups of six, consisting of the control group and the STZ-induced group. The rats in the STZ-induced group received 50 mg of STZ. The dose was expected to be sufficient to cause pancreatic cell damage (Goyal et al. 2016).

The kidneys were fixed in 10% formol saline and then dehydrated using graded alcohol at 70%, 80%, 95%, and 100% (2x). After being cleansed with xylol, the kidneys appeared clear. The next stage was embedding the organs in liquid paraffin or Paraplast at a temperature of ±60°C. Following the procedure was the casting (blocking) process using plastic molds and metal plates. The plastic mold was placed on a metal plate. A small amount of liquid Paraplast was poured into the molds, and then the organs were inserted and positioned in the Paraplast. The liquid Paraplast was then poured again until it covered the entire mold. Paraffin blocks that had been made were left to harden before further

processing. The tissue of the kidneys was then cut using a microtome with a thickness of 5 mm. The samples were then stained with hematoxylin-eosin (HE) and Masson's trichrome (Mondal 2017).

Renal histology was observed, particularly on the glomerulus and renal tubules (proximal and distal convoluted tubules in the cortex). Hematoxylin and eosin staining and Masson's trichrome staining were used to observe any increase in collagen (fibrosis). The observation was performed using 4x10 magnification with five fields of view, i.e., top left, bottom left, top right, bottom right, and middle. There would be 10x magnification in each field of view. Glomerulus and renal tubules would be observed with a magnification of 40x10 (Rezk et al. 2017).

The observed parts of the glomerulus included capillaries, podocytes, and mesangial tissue. Capillary observation was focused on endothelial cell damage. The degree of capillary endothelial cell damage was calculated in each field of view of the glomerulus. The calculation was carried out by determining the percentage of capillary endothelial cell damage, as indicated by pyknotic nuclei or the detachment of cells from the basement membrane (Mondal 2017). The calculation was performed using the following formula.

$$\frac{\Sigma \text{ damaged endothelial cells}}{\Sigma \text{ total endothelial cell}} \; x \; 100\%$$

The average percentage of capillary damage in one field of view was calculated by dividing the number of damaged capillaries in each glomerulus by the number of glomeruli and multiplied by 100% (Levey et al. 2014).

$$\frac{\Sigma \text{ percentage of glomerular capillary damage in each glomerulus}}{\Sigma \text{ glomerulus in 5 fields of view}} \; x100\%$$

Observation of podocytes was focused on the presence or absence of visceral thickening of Bowman's capsule and podocyte cell damage in the visceral Bowman's capsule. The visceral thickening of Bowman's capsule was determined by comparing the thickness of the membrane of Bowman's capsule to that of podocyte cells. Podocyte damage was characterized by pyknotic nuclei or cells detached from the basement membrane (Nagata 2016, Kopp et al. 2020).

The level of podocyte cell damage in each field of view of the glomerulus was calculated by determining the percentage of podocyte cell damage with the formula formula (Levey et al. 2014).

$$\frac{\Sigma \text{ damaged podocytes}}{\Sigma \text{ total podocytes}} \; x \; 100\%$$

The average podocyte cell damage in one field of view was calculated by dividing the percentage of podocyte cell damage in each glomerulus by the number of glomeruli and then multiplying the result by 100% (Levey et al. 2014).

$$\frac{\Sigma \text{ percentage of podocyte cell damage in each glomerulus}}{\Sigma \text{ glomerulus in 5 fields of view}} \; x100$$

The degree of damage to Bowman's capsule pars visceral was calculated by comparing the size of the podocyte with the basement membrane. If the podocyte was larger than the mass membrane, it would indicate that there was no thickening of the basement membrane (Inker et al. 2016). The degree of the damage was calculated by dividing the number of thickened Bowman's capsules in one field of view by the number of glomeruli observed, as seen in the formula below.

$$\frac{\Sigma \text{ thickening of Bowman's capsule pars visceral}}{\Sigma \text{ glomerulus observed in 5 fields of view}} \; x \; 100\%$$

In the observation of mesangial tissue, the tissue inflammation was characterized by inflammatory cell powder and mesangial cell density. The maximum damage in one field of view was recorded. In the mesangial network scoring system, A score of 0 would indicate the presence of <10 inflammatory cells. A score of 1 would indicate that 10–20 inflammatory cells were present. A score of 2 would indicate twenty to thirty inflammatory cells. Moreover, a score of 3 would indicate the presence of >30 inflammatory cells (Inker et al. 2016). The density of mesangial cells was calculated using the following formula.

$$\frac{\Sigma \text{ mesangial cells}}{\text{glomerular area}}$$

Damage to the proximal and distal convoluted tubules of the kidney is defined as tubular atrophy. This condition would be characterized by either pyknotic nuclei, vacuolization of the cytoplasm, shedding of epithelial cells, or thickening of the tubular basement membrane (Pourghasem et al. 2015). The average number of injuries observed in each field of view was then calculated. The percentage of tubular injuries was calculated using the following formula.

$$\frac{\Sigma \text{ tubule damaged}}{\Sigma \text{ total tubule in one field of view}} \; x100\%$$

Observation of fibrosis was performed in the renal cortex using Masson's trichrome staining. Descriptive observations were conducted on the glomerulus and renal tubules. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA). The data were tested for normality and

homogeneity using the Saphiro-Wilk test and the Levene test. Comparison between groups of each parameter was tested by the Chi-square test ($p<0.05$). If there was a significant difference between groups, it would be followed by the least significant difference (LSD) multiple comparison test (Furman 2015).

RESULTS

The percentage of morphological changes in endothelial cells and podocyte cells in the STZ-induced group increased compared to the control group. The number of morphological changes in the control group of endothelial cells was 3.11±3.90%, while in the STZ-induced group it was 6.01±5.35%. Podocyte cells in the control group underwent morphological changes of 2.96±3.32%, while the STZ-induced group experienced morphological changes of 6.17±2.32% (Table 1). The percentage of damage to each kidney component in the control group and the STZ-induced group did not differ significantly for the endothelium and podocytes ($p>0.05$). However, significant differences were shown in the tubules ($p<0.05$), as shown in Figure 1. The mesangial density was 0.0076±0.0013 in the control group and 0.0061±0.0008 in the STZ-induced group. A statistical test between the groups showed no significant difference ($p>0.05$), as shown in Figure 2.

Table 1. Value of damage to kidney components according to the test results.

Parameters	Control group	STZ-induced group
Endothelial cells (%)	3.11±3.90	6.01±5.35
Podocytes (%)	2.96±3.32	6.17±2.32
Tubules (%)	5.06±1.97	9.44±1.64*
Mesangial density (mean)	0.0076± 0.0013	0.0061±0.0008

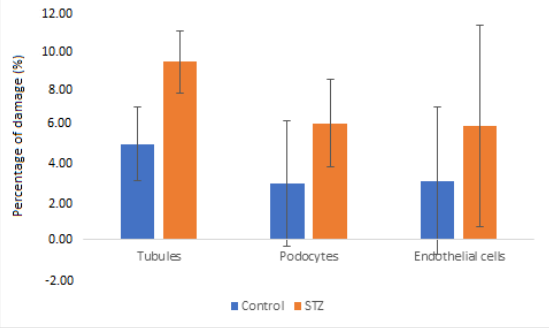

Figure 1. The percentage of damage to each kidney component in the control group and the STZ-induced group.

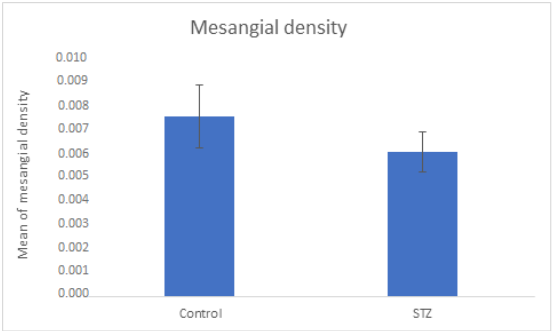

Figure 2. The mean of mesangial density in the control group and the STZ group.

Figure 3a shows the morphological changes of podocytes with pyknotic nuclei. The percentage of the changes increased, but there was no statistically significant difference ($p>0.05$). No inflammatory cells were found in the interstitial glomerulus in the control group or the STZ-induced group. In observing the thickening of the basement membrane, the membrane thickness was compared to the podocyte width. No basement membrane thickening was found.

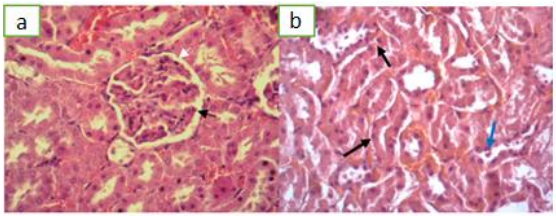

Figure 3. Hematoxylin-eosin staining of the STZ-induced group with 40x10 magnification: (a) In the glomerulus, pyknotic nuclei (black arrows) were seen in visceral podocytes, and normal nuclei (white arrows) were seen in the podocytes; (b) In the tubules, pyknotic nuclei (black arrows) were seen in tubular cells, and cells detached from the basement membrane (blue arrows).

Tubular examination using hematoxylin-eosin staining on the renal cortex showed an increase in the percentage of tubular cells with pyknotic nuclei of 9.44±1.64% in the STZ-induced group compared to 5.06±1.97% in the control group. A statistical test showed that there were significant differences between the two groups, with $p<0.05$. Microscopic observation of the proximal and distal convoluted tubules under 40x10 magnification revealed a significant difference in the STZ-induced group compared to the control group, with a p-value of 0.05. Pyknotic nuclei in the cells and separation of cells from the basement membrane indicated morphological changes in each group (Figure 3b).

The STZ-induced group displayed the most significant morphological changes.

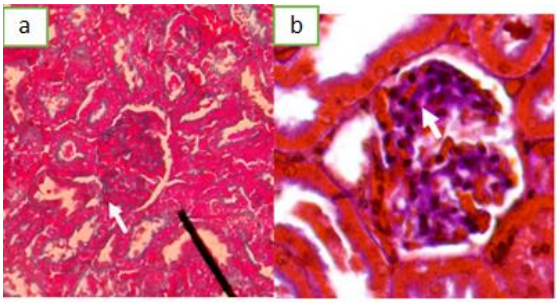

Figure 4. Masson's trichrome smear with 40x10 magnification: (a) In the control group, the glomerulus appeared blue only on the basement membrane; (b) In the STZ-induced group, it appeared in a faint blue color on the interstitial glomerulus (white arrows).

The glomerulus, tubules, and interstitial images appeared normal in the control group. The blue stain of collagen fibers was only found in the basement membrane. Glomerulus in the STZ-induced group showed a more diffuse blue stain than in the control group. Kidney samples from the control group showed blue staining in the renal capsule, tubular basement membrane, and glomerular capillary basement membrane (Figure 4a). In the STZ-induced group, blue-stained fibers were observed not only in the basement membrane of the glomerular capillaries but also in the glomerular mesangial tissue, although less clearly (Figure 4b).

DISCUSSION

This study examined the histological description of various kidney components, including podocytes, endothelium, basement membrane in the glomerulus, and tubules in the renal cortex. Uncontrolled hyperglycemia can lead to dysfunction in these components. It interferes with the permeability of the glomerular membrane, causing kidney damage over time (Anders et al. 2018).

The Animal Models of Diabetes Complications Consortium (AMDCC) of the United States of America (USA) recommends the use of standard animal models for developing renal complications of diabetes by induction of STZ. The recommended STZ administration is a low dose of 50 mg/kg for five consecutive days (Bayrasheva et al. 2016). According to the AMDCC, the use of STZ doses above 50 mg/kg can cause kidney damage not because of the effects of hyperglycemia but because of the direct effect of the STZ. Therefore, the 50 mg/kg dose in this study was expected to have an effect on the kidneys due to hyperglycemia and not because of pancreatic damage due to STZ toxicity.

Glomerular capillaries are a type of fenestrated capillary that allows water to pass rapidly through the capillaries. Capillaries consist of endothelium with flattened nuclei and are more heterochromatic (Mondal 2017). In this study, histological observations of endothelial damage at 40x10 magnification showed that the STZ-induced group had more changes in the morphology of endothelium compared to the control group. However, the statistical analysis showed no significant difference in each group ($p>0.05$). The observations revealed that the capillary endothelium had a flattened nucleus and a more heterochromatic staining pattern. Reactive oxygen species generated by hyperglycemia in the kidneys will increase nitrite oxide (NO) so that capillary damage can occur. This condition is characterized by endothelial dysfunction, which can be observed microscopically as pyknotic nuclei or separation of the basement membrane (Sol et al. 2020). This study determined that the effect of hyperglycemia on endothelial cells in the glomerulus was either the presence of pyknotic nuclei or the release of endothelial cells.

An increase in podocyte morphological changes may occur due to the presence of advanced glycation end products (AGEs). In kidneys with hyperglycemic conditions, AGEs contribute to an increase in reactive oxygen species. In addition, AGEs play an important role in apoptosis and the release of cells from the basement membrane (Reidy et al. 2014). An increase in podocyte damage in the STZ-induced group indicated elevated apoptotic conditions in the kidney. The damage contributed greatly to apoptosis and cell release from the basement membrane. In the control group, visceral pars podocytes were found to have relatively large cells with euchromatic nuclei. This suggests that hyperglycemia indirectly caused podocyte damage in the STZ-induced group, although the difference was not statistically significant (Lin & Susztak 2016).

Glomerular infiltration and basement membrane thickening are histological changes that can be observed in kidney damage due to diabetes. Hyperglycemia in the renal glomerulus can increase the expression of monocyte chemoattractant protein-1 (MCP-1), a chemokine that regulates the migration and infiltration of inflammatory cells such as macrophages (de M. Bandeira et al. 2013). In this study, no inflammatory cell infiltration was found in the glomerulus. According to a study by Goyal et al. (2016), the diabetogenic activity of STZ that can result in kidney injury was optimally administered in doses of 65 mg/kg. DNA damage and the infiltration of inflammatory cells such as

macrophages were possible with these doses. In this study, STZ injections were administered to the experimental animals only once. It was expected that the STZ injections would be sufficient to induce hyperglycemia and pancreatic cell damage. The duration of hyperglycemia may explain why there was no change in the glomerulus, given that the AMDCC recommends STZ administration for five consecutive days.

The method used in this study for determining the thickening of the basement membrane was by comparing the podocyte cells with the basement membrane. If the podocyte membrane was larger than the basement membrane, there would be no thickening of the basement membrane. The basement membranes in each group had the same pattern, and the podocytes were still larger than the basement membrane. In a number of earlier studies, different findings were obtained from observations using electron microscopy. The findings showed that there was thickening of the basement membranes (Kymioni Vasiliki-Maria et al. 2016, Rezk et al. 2017). A study by Sameni et al. (2016) reported that hyperglycemia-induced oxidative stress caused growth factors (such as transforming growth factor and tissue growth factor) to increase the extracellular matrix. As a result, the basement membranes became thicker. On the other hand, the results obtained from this study were limited due to a lack of tools, which made it difficult to objectively measure the thickness of the basement membrane

The renal tubule plays an important role in the progression of kidney disease due to diabetes. This component is frequently observed in studies of kidney damage (Giralt-López et al. 2020). The STZ-induced group in this study had significant morphological changes in the tubules. Observation of the cells in each group revealed the presence of pyknotic nuclei and the separation of cells from the basement membrane. Previous research showed that there were changes in the tubules of diabetic experimental animal models. In the histology of the tubules, there were flat epithelium and mononuclear cell filtration (Katsuda et al. 2015).

The increase in glucose due to STZ induction in the experimental animal model in this study proved that the initial state of diabetes had its first effect on the renal tubules. The glucose-induced activation of RAS will activate vascular endothelial growth factor. Along with reactive oxygen species, it will activate ornithine decarboxylase. Increased ornithine decarboxylase expression is known to cause renal tubular hypertrophy. Additionally, tubular hypertrophy and cell aging are caused by the induction of TGF-β and cyclin-dependent kinases that cause tubulointerstitial injury (Moonen et al. 2018).

Mesangial cell hypertrophy and fibroblast formation are also seen in kidneys that have been exposed to chronic hyperglycemia for a long time. TGF-β and extracellular matrix stimulation can be triggers for mesangial cell hypertrophy and fibroblast formation (Pourghasem et al. 2015). An analysis of mesangial density can be used to assess whether there is hypertrophy in the mesangial cells. The mesangial density is calculated by dividing the number of mesangial cells by the area of the glomerulus. The results of this study showed that there was no significant difference between the groups in terms of mesangial density in the glomerulus.

Masson's trichrome staining can be used to determine the presence or absence of collagen fibers in response to an increase in reactive oxygen species. This is important as it can lead to an increase in the production of extracellular matrix (Mondal 2017). The appearance of blue staining indicates collagen fibers. The glomerular mesangial tissue in the STZ-induced group showed blue-colored fibers. These findings suggest that the formation of excess extracellular matrix in the glomerulus in the STZ-induced group was due to an increased role of reactive oxygen species, which increased the extracellular matrix production via TGF-β (Sutariya et al. 2016).

Strength and limitations

Our study evaluated every component of the kidney that might be affected by increasing blood glucose levels using microscopy. This study used a scoring system that provided a validated outcome measure. However, microscopic examination and subjective assessment became the limitations of this study. Assessment of the histological aspects in this study involved two people, and each of them confirmed their findings. This study may inspire future studies to use improved scoring of the histological assessment to avoid subjectivity.

CONCLUSION

Hyperglycemia as a result of pancreatic β-cell damage in STZ-induced rats showed a significant difference only in the renal tubules. Other kidney components, such as endothelium, podocytes, and mesangial cells, showed an increase in damage but did not show any significant differences.

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Conflict of interest

None.

Ethical consideration

This study received ethical clearance from the Faculty of Medicine, Universitas Indonesia-Dr. Cipto Mangunkusumo National Central Public Hospital, Jakarta, Indonesia, with protocol No. 21-08-0850 on 16/08/2021.

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Author contribution

All authors contributed to the data collection, discussion of the content, and writing, reviewing, and editing of the manuscript before submission.

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Original Research Report

WORKLOAD INDICATORS OF STAFFING NEED (WISN) FOR THE REQUIRED ESTIMATION OF NURSING STAFF IN A HEMODIALYSIS UNIT

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ABSTRACT

The number of hemodialysis patients continues to increase almost every month, while the number of nurses in the Hemodialysis Unit at Rasyida Renal Specialist Hospital, Medan, Indonesia, remains constant. This situation has increased the nurses' workload and responsibilities. Therefore, it is necessary to examine the balance between the workload and the number of required nursing staff in the Hemodialysis Unit at Rasyida Renal Specialist Hospital. This study aimed to identify the optimal number of nursing staff required in the Hemodialysis Unit at the hospital in 2022. This study used a mixed-methods approach using the Workload Indicators of Staffing Need (WISN) method. This study included 44 nurses working in the Hemodialysis Unit at Rasyida Renal Specialist Hospital. Primary data included questionnaires, observations, and interviews during working hours, while secondary data included document reviews and staffing data. WISN calculations showed that the Hemodialysis Unit required a total of 65 nursing staff. In other words, an addition of 21 nursing staff was needed to meet the current workload. In conclusion, the current human resources available are not sufficient, and the workload is beyond the capacity of the nursing staff at the hospital.

Keywords: Health system; human resources; nursing personnel; workloads; workload indicators of staffing need (WISN)

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Highlights:
1. This article presents an understudied topic of nursing workloads in a hemodialysis unit and concludes that directly and indirectly productive activities occupied the majority of the nurses' working time.
2. An analysis using the Workload Indicators of Staffing Need produced a general report that may prompt re-evaluation of nursing staff requirements and policy-making for workload distribution.

INTRODUCTION

Human resource planning according to the needs and service functions in each unit, section, and department affects the success and development of a hospital (Elarabi & Johari 2014). Data from the Development and Empowerment Agency for Health Human Resources (Badan Pengembangan dan Pemberdayaan Sumber Daya Manusia Kesehatan/BPPSDMK) revealed that the percentage of nurses (29.66%) was the largest among other health workers in Indonesia as of December 2016. In 2016, the national ratio of registered nurses per 100,000 people was 113.40. The national rate in 2016 was significantly higher than in the previous year, although it did not reach the target for 2014, which was 158 per 100,000 people (Ministry of Health 2013). The 2019 goal was increased to 180 per 100,000 people. The evaluation of the nurse-to-patient ratio is important because a nurse is required to provide appropriate nursing care despite the heavy workload and responsibility (Destiani et al. 2020, Moghadam et al. 2021, Maghsoud et al. 2022). It should be noted that a heavy workload can result in work deviations. It is possible for a nurse's performance to decline, resulting in suboptimal nursing care (Kokoroko & Sanda 2019, Rifani & Djamil 2022).

In the late 1990s, the World Health Organization (WHO) developed the Workload Indicators of Staffing Need (WISN) method. This method

provides guidelines to estimate staffing needs, including population-to-staff ratio calculations (World Health Organization 2010). The Ministry of Health of the Republic of Indonesia has been using this method as a reference for calculating the required number of nursing staff since 2004. It has been legalized through the Decree of the Minister of Health Number 81/MENKES/SK/2004. Several studies, including Bonfim et al. (2016), Joarder et al. (2020), and Nuruzzaman et al. (2022), used the WISN method to quantitatively estimate nursing staff requirements based on activity standards and workloads. Furthermore, the WISN method was also used in a study to assess the imbalance in the distribution of nursing staff at Burdwan District Hospital, India. The average WISN results for the entire Burdwan District showed that only 35% of the required nurses were available, indicating a 65% shortage of nurses (Shivam et al. 2014).

The problem that frequently arises in nurse staffing is an imbalanced distribution of nurses. It may be because the directors have a difficult time identifying the workload in each unit (Spurgeon et al. 2012). If the problem is recognized, it is typically due to subjective complaints. The distribution of nurses has become one of the primary concerns of the nursing industry in order to provide better health programs and services to the Indonesian population (Gunawan 2016, Efendi et al. 2018). It is essential to provide prospective nurses with training and education to accommodate the demand for health personnel in hospitals and other types of health services. However, nurse staffing requirements, including the number of required nurses, are a prevalent issue in the health systems of many countries, especially in low- and middle-income countries (Martono et al. 2019, Frota et al. 2020, Efendi et al. 2022). Ekawati (2018) and Jayanti (2018), who conducted their studies in Yogyakarta and Medan, Indonesia, respectively, found a shortage of nursing staff according to the analysis results using the WISN method. There was a shortage of six nurses each in the Al-Kausar Inpatient Ward of Hospital X in Yogyakarta and the Inpatient Ward of Bunda Thamrin General Hospital, Medan, Indonesia. Only 72 nurses were available in the Inpatient Ward of Bunda Thamrin General Hospital, while 78 nurses were needed.

In research conducted by Pangaribuan (2015), it was concluded that the comparison of productive and non-productive time in the Hemodialysis Unit of Dr. Djasamen Saragih General Regional Hospital, Pematang Siantar, Indonesia, was 85%:15%. The findings indicated that the nurses in the Hemodialysis Unit had a heavy workload. The calculation of staffing requirements using the WISN showed that the required number of staff was 13 people. However, the nurses available were only 11 people, so there was a shortage of 2 nurses. In this study, the WISN method was also used to assess staffing requirements for nurses in the Hemodialysis Unit of Rasyida Renal Specialist Hospital, Medan, Indonesia. The idea for this research came from nurses' complaints that working in the Hemodialysis Unit entailed a relatively heavy workload because the number of patients was large and increasing every month while the number of nurses remained constant. This study was conducted during the COVID-19 pandemic, when the nurse staffing requirements in relation to the nurses' workload had never been evaluated. Therefore, the purpose of this study was to determine the actual number of nurses required in the Hemodialysis Unit of Rasyida Renal Specialist Hospital.

MATERIALS AND METHODS

The research was conducted at the Hemodialysis Unit of Rasyida Renal Specialist Hospital, Medan, Indonesia, from August to November 2022. In 1995, the hospital was originally a kidney and hypertension specialist clinic. In 2016, the hospital changed its name to Rasyida Renal Specialist Hospital, with class C classification, in order to meet the demand for improved and broader services in the fields of kidney and hypertension. The hospital has 69 active hemodialysis machines spread over six rooms. This research was a mixed-methods study that combined qualitative and quantitative methods using the Workload Indicators of Staff Need (WISN). The WISN is a method for calculating staffing requirements in healthcare facilities based on the actual workload in each work unit. The WISN method is highly dependent on the accuracy and completeness of workload data (McQuide et al. 2013). All active nurses in the Hemodialysis Unit of Rasyida Renal Specialist Hospital were included in this study. The sample consisted of 44 nurses, who were also the total population.

This study was conducted by paying attention to research ethics considerations, including: (1) Ethical approval by the Health Research Ethics Committee at Universitas Prima Indonesia, Medan, Indonesia; (2) Permission and recommendations from the Faculty of Public Health, Universitas Prima Indonesia, and Rasyida Renal Specialist Hospital were obtained prior to conducting the research; (3) All respondents provided informed consent; (4) Before distributing the consent forms, the researchers explained the research objectives to prospective respondents; (5) The researchers safeguarded the respondents' anonymity by omitting their names from the observation sheets; and (6) The researchers guarantee the confidentiality of all

information provided by respondents (Kieft et al. 2014).

Quantitative data were obtained using an observational approach and work sampling by observing the nurses' work activities. The amount of time they spent working on productive and non-productive activities indicated the workload categories. The data obtained were used to analyze labor requirements using the WISN method (Joarder et al. 2020). We also collected qualitative data by interviewing several respondents to support the results of the quantitative data analysis and to explore the respondents' responses in accordance with the objectives of this study. The respondents interviewed in this study were one human resources staff member, the head of the Hemodialysis Unit, and eight nurses of the Hemodialysis Unit.

Workload analysis was carried out to understand the description of the workload using observation, questionnaires, and interviews. The observed nursing activities were categorized as directly productive, indirectly productive, and non-productive. First, directly productive nursing activities are those directly related to patient care. Direct productive activities in the Hemodialysis Unit include the installation of hemodialysis equipment on patients, the administration of heparin injections, the measurement of blood pressure, the management of hemodialysis treatment, the explanation of hemodialysis procedures to new patients, and the observation of patients. Second, indirectly productive nursing activities include preparations and activities in complete nursing care that are not directly related to patient care. In the Hemodialysis unit, these tasks include activating the hemodialysis machine, filling in medical records, reusing the dialyzer, and disposing of infectious waste. Third, non-productive activities are those unrelated to nurses' duties and responsibilities, such as eating, praying, using the restroom, chatting, and preparing for personal hygiene (Spurgeon et al. 2012).

The variables observed in this study included available working time, work units, human resource categories, standard workloads, allowance standards, and staff requirements per work unit. Observation sheets were used in this study to measure the workload. As reported by the World Health Organization (2010), the formula for calculating available working time was A–(B+C+D+E)xF, with A representing working days, B representing annual leave, C representing education and training, D representing national holidays, E representing absence from work, and F representing working time. In order to determine the standard workloads, the amount of available work time was divided by the average time required to complete each task. Category allowance standards were observed to obtain data on support activities that are not directly related to or influenced by the quality or amount of the directly productive activities. The formula used for calculating allowance standards was dividing the total working time for each type of activity by the available working time. In addition, category allowance factors were also observed to determine the total number of nurses required for both directly productive health services and other activities. The formula for determining staff requirements was dividing the total workload for each workload component by each respective standard workload, then adding the allowance standard. A staff requirement ratio of <1 indicated that there were insufficient human resources.

RESULTS

The participants in this study were 44 practical nurses in the Hemodialysis Unit of Rasyida Renal Specialist Hospital in August 2022. All nurses in the unit had received hemodialysis training. Among the respondents, there were more male nurses than female nurses (23:21). Nurses aged <35 years were the majority (35:9), and nurses with a level 3 diploma were more common than those with a bachelor's degree (25:19), with an average of >3 years of experience working in the Hemodialysis Unit.

Table 1. Percentage of workload based on work sampling in the Hemodialysis Unit.

Types of activities	Percentage
Directly productive activities	36 %
Indirectly productive activities	46 %
Non-productive activities	18%
Total	100 %

Table 1 shows the observation results, which describe the types of activities carried out by nurses in the Hemodialysis Unit of Rasyida Renal Specialist Hospital. The nurses used their time mainly to carry out productive activities (82%), with 36% of their time spent on directly productive activities and 46% on indirectly productive activities. The proportion of their time spent on non-productive activities was only 18%.

Analysis of staffing requirements using the WISN

Workers in the Hemodialysis Unit must work six days per week in shifts. There were two shifts in a day, and each shift is 7 hours long. Nurses in the

Hemodialysis Unit of Rasyida Renal Specialist Hospital must have 312 working days per year. The policy in this hospital was that the staff had an annual leave of 14 days per year. Education and training were set for 20 hours per year, or 3 working days per year. According to the Joint Ministerial Decree in 2022, there were 16 days for national holidays and 4 days for collective leave. However, Rasyida Renal Specialist Hospital only provided 14 days of public holidays. As shown in Table 2, the available working time in the Hemodialysis Unit of Rasyida Renal Specialist Hospital was 1,932 hours per year, or 115,920 minutes per year.

Table 2. Available working time for nurses in the Hemodialysis Unit.

Code	Factors	Amount
A	Weekdays (6x52)	312 days/year
B	Annual leave	14 days/year
C	Education and training	3 days/year
D	Public holidays and collective leave	14 days/year
E	Absence from work	5 days/year
F	Working time	7 hours/day
Available working time = [A-(B+C+D+E)]×F		
1,932 hours/year or 115,920 minutes/year		

Table 3. Standard workloads for directly productive activities.

Workload components	t	AWT	SW
Installation of the device and administration of heparin injection	15	115,920	7,728
Measurement of blood pressure	7	115,920	16,560
Patient observation	8	115,920	14,490
Observation of patients with complications	6	115,920	19,320
Consultation with patients regarding their complaints	6	115,920	19,320
Treatment of patients with complications	14	115,920	8,280
Hemapoethrin injection for patients with indications	5	115,920	23,184
Injection of medications or multivitamins for patients with indications	3	115,920	38,640
Finishing hemodialysis treatment	8	115,920	14,490
Explanation of the hemodialysis procedure for new patients	8	115,920	14,490

Notes: Time average (t), available working time (AWT), and standard workloads (SW) were measured in minutes.

Table 4. Standard workloads for indirectly productive activities.

Workload components	t	AWT	SW
Activation of the hemodialysis machine	13	115,920	8,916
Preparation of tools and materials for hemodialysis	14	115,920	8,280
Reuse of dialyzers	115	115,920	1008
Preparation of the bed, including the bed sheets and pillows	4	115,920	28,980
Preparation of acid and new bicarbonate	5	115,920	23,184
Disposal of infectious waste	3	115,920	38,640
Reactivation of the machine if a malfunction occurs	6	115,920	19,320
Logistics management in the warehouse	10	115,920	11,592
Filing medical records	22	115,920	5,269
Data entry in the hospital management information system	20	115,920	5,796
Preparation of materials for patients	4	115,920	28,980
Preparation of intravenous (IV) injections for patients	5	115,920	23,184
Folding dirty linens	3	115,920	38,640

Notes: Time average (t), available working time (AWT), and standard workloads (SW) were measured in minutes.

Table 5. Standard workloads for non-productive activities.

Workload components	t	AWT	SW
Meal periods	19	115,920	6.101
Restroom breaks	7	115,920	16,560
Personal time (prayers, phone calls)	12	115,920	9,660
Chatting	13	115,920	8,916
Personal preparations	9	115,920	12,880

Notes: Time average (t), available working time (AWT), and standard workloads (SW) were measured in minutes.

Table 6 shows the percentage of category allowance standards for each type of activity based on the working time in the Hemodialysis Unit of Rasyida Renal Specialist Hospital. The nurses spent most of their working time on indirectly productive activities (61.5%). As shown in Table 7, the category allowance factor was 0.061. The largest proportion of the nurses' time for indirectly productive activities was spent on preparing consumables. This task required about 10 minutes, or 2,880 minutes a year.

Table 6. Percentage of category allowance standards for each type of activity.

Types of activities	Total time (minutes)	Percentage
Directly productive activities	80	22%
Indirectly productive activities	224	61.5%
Non-productive activities	60	16.5%
Total	364	100%

The analysis results of staff requirements using the WISN method revealed that the required number of nurses was 65.131 people, or rounded up to 65 people. The actual number of available nurses in the Hemodialysis Unit of Rasyida Renal Specialist Hospital was 44. Therefore, there was a shortage of 21 people on the nursing staff. The difference between the required number of nurses and the actual number of nurses in the Hemodialysis Unit produced a ratio of staff requirements. Comparison of these numbers resulted in a ratio of 0.67, indicating that the staff requirements were not met. The existing number of nurses in the Hemodialysis Unit was not sufficient.

Table 7. Category allowance factors for nursing staff in the Hemodialysis Unit of Rasyida Renal Specialist Hospital.

Workload components	t	AWT	CAF (t/AWT)
Nursing audit meeting	1,440	115,920	0.012
Arrangement of drugs and equipment	1,440	115,920	0.012
Arrangemen of essential consumables	2,880	115,920	0.025
Reecording and reporting	1,440	115,920	0.012
Total			0.061

t: Time average (minutes per year); AWT: Available working time; CAF: category allowance factor (CAF).

DISCUSSION

The work activities of nurses in the Hemodialysis Unit of Rasyida Renal Specialist Hospital showed that directly and indirectly productive activities for care services required more time than non-productive activities. Indirectly productive activities include documentation, communication, meetings, reporting, and administrative work (Sharma & Rani 2020, Yulliswandi et al. 2022,

Table 8. Analysis of the staff requirements in the Hemodialysis Unit of Rasyida Renal Specialist Hospital.

Workload components	SW	Required nurses
Installation of the device and administration of heparin injection	7,728	5
Measurement of blood pressure	16,560	2.3
Patient observation	14,490	2.7
Observation of patients with complications	19,320	2
Consultation with patients regarding their complaints	19,320	2
Treatment of patients with complications	8,280	2.3
Hemapoethrin injection for patients with indications	23,184	1.6
Injection of medications or multivitamins for patients with indications	38,640	0.27
Finishing hemodialysis treatment	14,490	2.7
Explanation of the hemodialysis procedure for new patients	14,490	0.9
Activation of the hemodialysis machine	8,916	4.3
Preparation of tools and materials for hemodialysis	8,280	4.7
Reuse of dialyzers	1,008	0.3
Preparation of the bed, including the bed sheets and pillows	28,980	1.3
Preparation of acid and new bicarbonate	23,184	0.1
Disposal of infectious waste	38,640	0.3
Reactivation of the machine if a malfunction occurs	19,320	0.5
Logistics management in the warehouse	11,592	1.7
Filing medical records	5,269	7
Data entry in the hospital management information system	5,796	6
Preparation of materials for patients	28,980	0.6
Preparation of intravenous (IV) injections for patients	23,184	0.8
Folding dirty linens	38,640	0.5
Meal periods	6,101	6
Restroom breaks	16,560	0.8
Personal time (prayers, phone calls)	9,660	4
Chatting	8,916	1.4
Personal hygiene preparations	12,880	3
Subtotal		65.07
Category allowance factor		0.061
Total		65,131

Note: Standard workloads (SW) is the quantity of workload for a year per workload component.

Hossny 2022). Indirectly productive activities are important and take up a great proportion of nurses' workload. However, nurses are most satisfied when they can devote more time to directly productive patient care activities (Raeissi et al. 2015). This study showed that nursing activities in the Hemodialysis Unit were quite busy. There were similarities between this study and another study conducted in a radiology unit where nurses spent more time on directly and indirectly productive care activities compared to non-productive activities (Farrasizdihar et al. 2021). Productive activities are the main activities in nurses' duties and responsibilities. Therefore, the nurses on duty are more occupied with carrying out productive activities, both directly and indirectly, than non-productive activities (Kieft et al. 2014, Leal & Melo 2018).

In hospital settings, a high workload poses a danger to patient safety. Unwanted occurrences may happen and endanger patients in precarious situations (Vaismoradi et al. 2020, Govasli & Solvoll 2020). One of the solutions that can prevent undesirable occurrences is meeting the staff requirements or hiring additional nurses. This solution is anticipated because patient visits tend to increase every month and will continue to rise in the coming years.

The findings of this study revealed that nurses in the Hemodialysis Unit of Rasyida Renal Specialist Hospital spent more time on indirectly productive activities than directly productive activities, with a comparison of 61.5% and 22%, respectively. The time spent on non-productive activities was only 16.5%. Indirectly productive activities required a great proportion of the nurses' workload because of the long process of reusing the dialyzer. The total productive time (83.5%) in the Hemodialysis Unit for one shift passed the optimal point (Gunawan 2016). A study showed that the optimal proportion of working time for productive activities is 80%. Therefore, it was necessary to consider hiring additional nurses.

In the analysis of working time, it was found that nurses in the Hemodialysis Unit had at least one day off per week. Nurses with less than three years of experience had only 12 days off per year, while those with more than three years of experience had 14 days off. In the Hemodialysis Unit, there were no minimum hours of training or seminars per year. However, there was a plan in 2022 to start allocating 20 hours per year, or three working days per year, for education and training. Another study found that the optimal working hours for nurses are divided into three shifts. Nurses are entitled to eight days off per month and three national holidays per year (Sharma & Rani 2020).

According to the results of the overall calculation, it was found that the amount of available working time for nurses in the Hemodialysis Unit was 115,920 minutes per year. However, a study conducted by (Farrasizdihar et al. 2021) found a greater amount of available working time in a radiology unit of a hospital. According to the ministerial decree of the Ministry of Manpower and Transmigration, the definition of overtime is working that exceeds the recommended working hours. Employers should adhere to the recommended working hours, i.e., 7 hours a day and 40 hours a week for 6 working days a week, or 8 hours a day and 40 hours a week for 5 working days a week. The working hours of nurses in the Hemodialysis Unit did not exceed the maximum working hours for workers with six working days a week. However, nurses on the afternoon shift might still have to work overtime. The interview showed that the nurses received bonuses or incentives from the hospital as rewards for providing excellent hemodialysis care services.

In this study, the observations and interviews showed a category allowance factor of 0.061. The working components that affected this factor included nursing audit meetings, preparation of medicines and equipment, preparation of consumables, and reporting. The category allowance factor in this study was smaller than in previous studies that showed category allowance factors of 0.183 and 0.39 (Ekawati 2018, Farrasizdihar et al. 2021). The small category allowance factor in this study might be because there were not many activities conducted outside of the Hemodialysis Unit of Rasyida Renal Specialist Hospital. The hospital did not set a schedule for all nurses to take part in these activities, but only for several representative nurses. It was done to prevent the Hemodialysis Unit rooms from being vacant if the nurses were away for activities outside the hospital. One skilled nurse is required for every two hemodialysis machines per shift. The nursing staff requirements for two shifts are 70 nurses (Afzal et al. 2021). There were only 44 nurses and 69 hemodialysis machines in the Hemodialysis Unit of Rasyida Renal Specialist Hospital. Each nurse in the Hemodialysis Unit should operate about three hemodialysis machines. Therefore, the number of nurses in the Hemodialysis Unit was insufficient.

The results of a previous study showed that there was a shortage of six nurses at Bunda Thamrin General Hospital. The number of available nurses in the hospital was only 72, while 78 nurses were required to equally distribute the nurses' workload (Jayanti 2018). In another study, the obtained results also showed insufficient human resources for the existing workloads, with a staff requirement ratio of 0.7. There were only 15 nurses in the

inpatient room of a hospital in Yogyakarta, Indonesia, while the required number of nurses was 21 (Ekawati 2018). In contrast, a study by Farrasizdihar et al. (2021) in a hospital's radiology department found that there was enough staff available. Both the existing and required number of nurses in the unit were 15 people, so the staff requirement ratio was 1.00. The staff requirement ratio in this study was 0.67. The low ratio indicated that the Hemodialysis Unit of Rasyida Renal Specialist Hospital had a shortage of nurses. This situation can affect the care services provided by the nurses, such as the increased potential for medication errors. This study suggests that the management of Rasyida Renal Specialist Hospital should consider planning a more balanced staffing by referring to the analysis results of staff requirements.

Strength and limitations

The strength of this study was that it provided insight into the heavy workloads of nurses that are often left unnoticed. This study may also serve as a prompt for a re-evaluation of staff requirements in the hospital. In addition, the data in this study were collected not only through interviews but also through observations, so the data obtained were objective. The respondents completed the instrument questionnaires under the supervision of the researchers in order to obtain accurate data. However, due to time constraints, it was challenging to gather information regarding current circumstances and standard operating procedures in the Hemodialysis Unit.

CONCLUSION

According to the Workload Indicators of Staffing Need (WISN) calculations, the current human resources at Rasyida Renal Specialist Hospital are not sufficient. The heavy workload, especially related to indirect productive activities, emphasizes the need for additional nursing staff to balance the workload. In addition, nursing management should consider the standard workload as well as implement effective and efficient strategies for planning and scheduling to estimate production costs, including the cost of nursing staff. The use of the WISN method is recommended to determine the number of staff and to reevaluate the distribution of nurses in both the Hemodialysis Unit and the inpatient rooms according to the specific requirements of each area.

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Conflict of interest

None.

Ethical consideration

This study was approved by the board of the Ethical Committee of Universitas Prima Indonesia, Medan, Indonesia, as indicated by registration No. 034/KEPK/UNPRI/2022 on 30/9/2022.

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None.

Author contribution

FCW contributed to the conception and design of this study, analyzed and interpreted the data, drafted the article, and collected and assembled the data. SLRN and CNG contributed to the critical revisions of the article for important intellectual content and provided the study materials and statistical expertise.

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Original Research Report

IMPROVEMENT OF CADRES' SKILLS AND KNOWLEDGE TO PROVIDE COMPREHENSIVE HEALTH SERVICES FOR THE ELDERLY

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ABSTRACT

Numerous issues have arisen as a result of the large size and rapid growth of the elderly population, attesting to the need for serious efforts from all sectors to enhance their welfare. However, the health cadres at the Integrated Health Post for the Elderly (*Pos Layanan Terpadu Lanjut Usia*/ Posyandu Lansia) lacked the training that would have equipped them with the necessary knowledge and skills to carry out their duties. This study aimed to evaluate the significance of training for enhancing the cadres' knowledge and skills in providing comprehensive health services for the elderly. This study was quasi-experimental research with a pre-test and post-test design. The data analysis was performed using the Shapiro-Wilk test ($p>0.05$) and the Wilcoxon test ($p<0.05$). A total of 30 health cadres at Songgon Community Health Center were measured for their levels of knowledge and skills before and after the training. The training included filling out health evaluation charts, providing leaflets containing health counseling materials for the elderly, practicing exercise for the elderly, and singing an elderly marching song. Before filling out the health evaluation chart, the cadres had to measure the elderly's degree of independence as well as their mental, emotional, and nutritional status. The cadres also had to measure the weight, height, and blood pressure of the elderly. Prior to training, the cadres' knowledge of hospitalization referrals, independence, and physical exercise for the elderly was limited. The cadres' skills in filling out health evaluation charts, assessing mental, emotional, and nutritional status, measuring blood pressure, and instructing physical exercise were also inadequate. There was a significant difference in the knowledge and skills of the health cadres before and after training ($p<0.05$). In conclusion, there was an improvement in the health cadres' knowledge and skills regarding comprehensive health services for the elderly at the Posyandu Lansia.

Keywords: Skills; health cadres; Integrated Health Post for the Elderly (Posyandu Lansia); comprehensive health services for the elderly; public health

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Highlights:
1. Due to the increasing elderly population in Indonesia, this study must address the knowledge and skill gaps among health cadres in a number of Integrated Health Posts for the Elderly (*Pos Layanan Terpadu Lanjut Usia*/ Posyandu Lansia).
2. Through this study, the health cadres increased their knowledge and skills, enabling them to provide comprehensive health services for the elderly.

INTRODUCTION

The success of national development has an impact on increasing life expectancy. The life expectancy of the Indonesian population in 2021 was estimated to be 69.67 years for men and 73.55 years for women (Statistics Indonesia 2022). However, increasing life expectancy does not only occur in Indonesia, as population aging is a global trend in this millennium. The worldwide proportion of the elderly population aged 60 years and over is growing very rapidly compared to other age groups. The global growth of the elderly population from 2000 to 2025 is expected to double, from 606

million (10% of the world's total population) to 1.2 billion. In developing countries, the increase in the elderly population is greater than in developed countries. Indonesia is a country with an aging population structure, where the elderly population was projected to be 27.08 million people, or 9.99% of the total population (Statistics Indonesia 2017).

The elderly experience health, economic, social, spiritual, and legal challenges. They frequently suffer from geriatric syndromes, degenerative diseases, infectious diseases, and trauma. Geriatric syndromes are a group of symptoms that arise as a result of the aging process (Olsen et al. 2016). The symptoms of geriatric syndromes are known as the 5 Bs in Javanese, i.e., *blaur* (presbyopia), *budek* (prebiacusis), *beser* (incontinence), *bungkuk* (osteoporosis), and *bingung* (dementia). Degenerative diseases prevalent among the elderly are hypertension (63.5%), diabetes mellitus (5.7%), heart disease (4.5%), stroke (4.4%), kidney disorders (0.8%), and cancer (0.4%). The most prevalent infectious diseases among the elderly are acute respiratory infections (28.1%), diarrhea (24%), pneumonia (16.3%), and pulmonary tuberculosis (2.6%) (Ministry of Health of the Republic of Indonesia 2019).

According to Law of the Republic of Indonesia No. 13 of 1998 Concerning the Welfare of the Elderly, the health of the elderly is a state of body, spiritual, and social well-being that allows them to live socially and economically productive lives. The elderly cannot be excluded from public healthcare, as every effort to improve the public health status is an investment in the development of the country (Djamhari et al. 2020). In addition, Act of the Republic of Indonesia No. 36 of 2009 on Health mandates that efforts to improve and maintain public health must include the elderly and be conducted in accordance with nondiscriminatory, participatory, and sustainable principles. The principle of nondiscrimination requires that all individuals, including the elderly, have access to health services (Wahono et al. 2023). The large size and rapid growth of the elderly population contribute to a variety of problems, so all sectors must devote significant resources that will improve their welfare.

In Indonesia, the Community Health Center plays an important role in the health development of its respective service area. The Community Health Center is responsible for coordinating community health initiatives in order to increase the community's awareness, willingness, and capacity to live a healthy lifestyle and obtain optimal health status (Harahap 2021). The Community Health Center organizes the Integrated Health Post for the Elderly, which is also known as *Pos Layanan Terpadu Lanjut Usia* (Posyandu Lansia) in Indonesian. Posyandu Lansia is a form of community participation in the provision of health services for the elderly. In Posyandu Lansia, the community conducts routine health examinations with the participation of health cadres. However, health services at Posyandu Lansia had not been implemented optimally in a number of areas (Arsyati & Chandra 2020). One of the causes was the lack of knowledge and skills of the cadres in providing health services to the elderly. Therefore, training is essential to enhance the knowledge and skills of the cadres in providing comprehensive health services at Posyandu Lansia. This study aimed to analyze the differences in the cadres' knowledge and skills before and after the training. After the training, the cadres are expected to contribute to the improvement of the health status, productivity, and independence of the elderly population.

MATERIALS AND METHODS

The present study was conducted as quasi-experimental research with a pre-test and post-test design. The intervention in this study was training the cadres at Posyandu Lansia to provide comprehensive health services. A questionnaire was used to conduct pre- and post-tests on the cadres before and after the intervention to assess their level of knowledge and skills (Alessandri et al. 2017, Stratton 2019). This study was conducted at the Songgon Community Health Center in Banyuwangi, Indonesia, in September 2022. The service area of Songgon Community Health Center covers nine villages with 21,463 elderly citizens. Songgon Community Health Center is responsible for coordinating 11 Posyandu Lansia with 30 health cadres. The Community Service Team from the Department of Public Health and Preventive Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, conducted a two-day training in the hall of the Songgon Community Health Center. This research received ethical approval from the Health Research Ethics Committee, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, with registration No. 180/EC/KEPK/FKUA/2022 on 19/09/2022.

The training session involved the participation of 30 health cadres. The seminar materials included a definition of the elderly, problems among the elderly, health concerns among the elderly (geriatric syndromes), comprehensive health services, the role of the health cadres, the role of Posyandu Lansia, the five desks for Posyandu Lansia, the frequency of health services in Posyandu Lansia, and health evaluation charts for the elderly (Dewi et al. 2018). The health cadres were trained to fill out health evaluation charts for the elderly. Initially,

they must measure the degree of elderly independence, mental and emotional state, nutritional status, weight, height, and blood pressure. Additionally, they were assigned the responsibility of distributing leaflets that contained health counseling materials, practicing physical exercise, and performing the marching song for the elderly. The participants were measured for their levels of knowledge and skills about comprehensive health services for the elderly by filling out questionnaires before and after the training (Zadworna-Cieślak 2020).

Descriptive and analytic statistics were used in this study. As the data were ordinal, the descriptive statistics are presented in tables of percentage frequency distribution, as well as a central tendency in the form of range (min-max) and median (Vetter 2017). The data were analyzed using the Shapiro-Wilk test to assess the normal distribution of the data, with a normal distribution indicated by $p>0.05$. It was then followed by the Wilcoxon test to determine whether there was any difference between the cadres' pre- and post-training knowledge and skills, with a significance of $p<0.05$ (Taheri & Hesamian 2013). The data were statistically analyzed using SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, Ill., USA).

RESULTS

Table 1 presents the characteristics of the health cadres at Posyandu Lansia of Songgon Integrated Health Care Center. The mean age of the cadres was 36 years. The majority of cadres were housewives with a high school degree. The Sedap Malam Posyandu had the highest number of health cadres.

Prior to the training, the knowledge of the health cadres was severely insufficient, especially in regards to blood pressure tests (100%), health evaluation (96.7%), elderly quality of life (96.7%), the definition of the elderly (93.3%), and elderly problems (100%), as shown in Table 2. In addition, the knowledge of the health cadres was limited in regards to hospitalization referrals (20%), physical exercise frequency (23.3%), elderly independence (30%), elderly productivity (43.3%), and elderly health concerns (46.7%).

After the training, there was an increase in the health cadres' knowledge, especially regarding blood pressure tests (100%), elderly health evaluation (100%), elderly quality of life (100%), the definition of the elderly (100%), and elderly problems (100%). However, several knowledge areas required further improvement, including hospitalization referrals (43.3%), the frequency of physical exercise (33.3%), elderly independence (33.3%), elderly productivity (83.3%), and elderly health concerns (70%).

Table 1. Characteristics of the health cadres.

Characteristics	n	%
Age (years)		
Mean±SD	36±8.83	
Education		
Elementary school	5	16.7
Junior high school	9	30
Senior high school	16	53.3
Occupation		
Housewife	23	76.7
Entrepreneur	6	20
Administrative worker	1	3.3
Posyandu names		
Arum Senja	1	3.3
Balak	2	6.7
Bayu	3	10
Beringin	3	10
Dahlia	2	6.7
Parikuning	3	10
Sedap Malam	5	16.7
Sragi	3	10
Sri Utomo	4	13.3
Sumberbulu	3	10
Teratai	1	3.3

Table 2. Distribution of the cadres' correct answers for the knowledge evaluation.

Knowledge	Pre [n (%)]	Post [n (%)]
Definition of the elderly	28 (93.3%)	30 (100%)
Elderly problems	27 (90.0%)	30 (100%)
Elderly health concerns	14 (46.7%)	21 (70.0%)
Elderly productivity	13 (43.3%)	25 (83.3%)
Frequency of exercise	7 (23.3%)	10 (33.3%)
Elderly independence	9 (30.0%)	10 (33.3%)
Blood pressure tests	30 (100%)	30 (100%)
Health evaluation	29 (96.7%)	30 (100%)
Elderly quality of life	29 (96.7%)	30 (100%)
Hospitalization referrals	6 (20.0%)	13 (43.3%)

Prior to the training, the cadres' skills were already sufficient in regards to measuring weight and height (100%) and providing health education (93.3%), as shown in Table 3. However, the cadres' lacked the skills for filling out health evaluation charts (30%), measuring nutritional status (40%), measuring emotional status (40%), instructing physical exercise (46.7%), and measuring blood pressure (50%).

After the training, there was an increase in the cadres' skills, particularly in filling out health evaluation charts (33.3%), measuring nutritional

status (86.7%), measuring emotional status (66.7%), and instructing physical exercise (76.7%). However, the cadres' skill to measure blood pressure decreased to 46.7%.

Table 3. Distribution of the cadres' correct answers for the skills evaluation.

Skills	Pre [n (%)]	Post [n (%)]
Filling out health evaluation charts	9 (30.0%)	10 (33.3%)
Measuring independence	22 (73.3%)	28 (93.3%)
Measuring blood pressure	15 (50.0%)	14 (46.7%)
Measuring weight	30 (100%)	30 (100%)
Measuring height	30 (100%)	30 (100%)
Measuring nutritional status	12 (40.0%)	26 (86.7%)
Measuring emotional status	12 (40.0%)	20 (66.7%)
Providing health education	28 (93.3%)	28 (93.3%)
Instructing physical exercise	14 (46.7%)	23 (76.7%)
Determining hospitalization refferals	18 (60.0%)	24 (80.0%)

The differences between pre-training and post-training knowledge and skills were analyzed. The normality test was used to determine whether or not the data were normally distributed. Table 4 shows the normality test results, with $p<0.001$ for both knowledge and skills variables.

Table 4. The normality test results of the differences between pre- and post-training knowledge and skills.

Variables	n	p
Knowledge	30	<0.001
Skill	30	<0.001

Table 5. The differences between pre- and post-training knowledge and skills.

Variables	Median (min–max)	Median (min–max)	p
Knowledge			
Pre	6.5 (1–9)	1 (0–6)	<0.001
Post	8 (5–10)		
Skills			
Pre	7 (2–10)	1 (0–5)	<0.001
Post	8 (3–10)		

The results of the Shapiro-Wilk test indicated that the differences between pre- and post-training knowledge and skills were not normally distributed ($p<0.05$). Therefore, the data analysis was followed by the Wilcoxon test.

The results of the Wilcoxon test are presented in Table 5. The analysis results revealed a statistically significant difference in the level of knowledge among the cadres prior to and following the training ($p<0.05$). Similarly, there was a notable difference observed in the skills of the cadres prior to and following the training.

DISCUSSION

The World Health Organization has categorized the elderly age group into three distinct subgroups. The category of the younger elderly comprises individuals aged 60–74 years, while the middle portion of the category comprises those aged 75–90 years. The older subgroup comprises individuals who are >90 years old (Lee et al. 2018). On the other hand, the Ministry of Health of the Republic of Indonesia has classified the elderly age group into three subgroups. These subgroups include the pre-senior subgroup consisting of individuals aged 45–59 years, the senior subgroup consisting of those aged 60–70 years, and the elderly subgroup with higher risk that includes individuals above 90 years of age (Sumiati et al. 2022).

There is no way to avoid aging, but it can be transformed into a healthy process through the maintenance and improvement of physical and mental health, independence, and quality of life. The aging process varies from person to person due to genetic, lifestyle, and health factors. The elderly experience progressive generalized functional impairment, a loss of adaptive stress responses, and an increased risk of age-related diseases (Hubbard et al. 2013, Levine 2013). Physical, psychological, and social conditions can influence the health and quality of life of the elderly.

In this study, the cadres' knowledge of health issues affecting the elderly improved from 46.7% to 70% after training (Table 2). This knowledge is crucial because elderly individuals are susceptible to developing health issues. Several health issues that are prevalent among the elderly are hypertension (63.5%), diabetes mellitus (5.7%), heart disease (4.5%), and stroke (4.4%) (Ministry of Health of the Republic of Indonesia 2019). Diabetes mellitus was recognized as the most prevalent health issue in the cadres' working area.

Comprehensive health services for the elderly include promotive, preventive, curative, and rehabilitative efforts. Promotive health services aim to revive the elderly's spirit of life so that they feel

valued and remain useful to themselves, their families, and the community. Preventive health services are intended to reduce the likelihood of age-related diseases that lead to complications. Curative health services are multidisciplinary, science-based endeavors to treat the elderly. Rehabilitative health services consist of efforts to restore the diminished function of body organs, for example, by wearing glasses, hearing aids, or dentures (Wendimagegn & Bezuidenhout 2019).

Posyandu Lansia is a forum for providing health services for the elderly. The community within Posyandu Lansia's service area is responsible for carrying out the formation and implementation processes. The government, non-governmental organizations, private sector, and social organizations play a role in the development of Posyandu Lansia, with a focus on promotive and preventative health services (Ilyas 2017). In addition to health services, Posyandu Lansia can provide social services, religious services, education, skills, exercise, arts, culture, and other services required by the elderly to improve their quality of life through enhanced health and welfare. The establishment of Posyandu Lansia facilitates elderly individuals becoming more active and developing their potential (Frisca et al. 2020).

Posyandu Lansia incorporates the utilization of five desks to represent the sequential process involved in delivering healthcare services. Desk 1 is designated for the purpose of registration. Desk 2 serves the purpose of examining weight and height measurements, determining body mass index (BMI), and documenting the results on the health evaluation chart. Desk 3 is designated for the purpose of conducting basic examinations and administering treatments such as blood pressure, blood sugar levels, hemoglobin levels, and vitamin supplements. Desk 4 is designated for the purpose of conducting counseling activities, specifically pertaining to subjects such as health, nutrition, and welfare. Desk 5 serves the purpose of delivering information and facilitating social activities, including the provision of additional food, capital assistance, mentorship, and other relevant forms of support as required (Nikmah & Khomsatun 2020, Harahap 2021). In this study, the cadres' skills to assess nutritional status increased from 40% before training to 86.7% after training (Table 3). The cadres were already skilled in measuring weight and height but had not been able to determine the nutritional status of the elderly based on the health evaluation chart. However, the cadres' skills to measure blood pressure remained low (50%) because they rarely worked at Desk 3. Health professionals assisted in carrying out the basic examinations and treatments.

In this study, Posyandu Lansia provided health services at least once per month. However, it was suggested that physical activity and religious study be performed at least once per week. The cadres' knowledge on the frequency of physical exercise slightly improved from 23.3% before training to 33.3% after training. The cadres believed that physical exercise for the elderly only needed to be carried out at the same time as the Posyandu Lansia health services, which were once per month. Generally, the elderly exercised prior to the start of health services. However, the elderly should have exercised at least once a week (Mardius & Astuti 2017).

The health evaluation chart for the elderly is a tool for recording the physical, mental, and emotional health conditions of elderly individuals. Posyandu Lansia records information on the elderly's health evaluation chart, which can be used to monitor and evaluate their health progress. It is also possible to monitor the degree of independence, emotional and mental conditions, blood pressure, nutritional status, and fasting blood sugar levels of the elderly through the health evaluation chart (Fatmah & Nasution 2012, Kusumawardani & Andanawarih 2018). In this study, the cadres' skill in filling out health evaluation charts for the elderly slightly improved from 30% to 33.3%. The cadres' skill in assessing the mental and emotional status of the elderly was limited (40%) but improved after training (66.7%).

By using the health evaluation chart, the cadres should be able to determine whether an elderly individual needs to be referred to a hospital (Riasmini 2021). Although the cadres' knowledge of hospitalization referrals was limited (20%) prior to training, it increased after training (43.3%). The cadres' knowledge and skills regarding the independence of the elderly increased from 30% to 33.3% and from 73% to 93%, respectively. Approximately 50% of the cadres have completed their education at the elementary and junior high school levels. The potential influence of the education levels of the cadres on their deficiency in knowledge and skills may be considered.

Strength and limitations

This study can raise awareness of the significance of training for establishing sustainable community services at Posyandu Lansia. This study may suggest a recommendation that health cadres and health workers participate in training regularly to increase their capacity and enable them to provide comprehensive health services for the elderly. However, the long-term impact of the training on the cadres' skills and knowledge could not be concluded, as this study only performed the intervention once.

CONCLUSION

The training conducted at the Posyandu Lansia of Songgon Community Health Center, Banyuwangi, Indonesia, led to a significant improvement in the cadres' knowledge and skills regarding comprehensive health services for the elderly. Prior to the training, the cadres had inadequate knowledge and skills in the frequency of physical exercise, the independence of the elderly, hospitalization referrals, filling out health evaluation charts, measuring blood pressure, assessing emotional and mental status, and instructing physical exercise. It is anticipated that the enhancement of the cadres' knowledge and skills will facilitate the implementation of comprehensive health services to promote healthy aging and the well-being of the elderly.

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Conflict of interest

None.

Ethical consideration

This research had received ethical approval from the Health Research Ethics Committee, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, with registration No. 180/EC/KEPK/FKUA/2022 on 19/09/2022.

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Author contribution

LDj contributed to the conceptualization, study design, data collection, data analysis, data interpretation, intervention, supervision, manuscript writing, and content revision. LDe contributed to the conceptualization, study design, data analysis, manuscript writing, and content revision. S contributed to the conceptualization, study design, manuscript writing, and content revision. NNB contributed to data collection and documentation. FN contributed to data collection and administration. SP contributed to data collection, intervention, and supervision.

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Original Research Report

THE EFFECTS OF PURPLE SWEET POTATO (*Ipomoea batatas* L.) ETHANOL EXTRACT ON BLADDER UROTHELIAL LAYER AND SMOOTH MUSCLE THICKNESSES IN MENOPAUSAL FEMALE WISTAR RATS

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ABSTRACT

Postmenopausal women experience estrogen hormone deficiency, which can cause thinning of the smooth muscle and urothelial layer of the bladder, leading to lower urinary tract symptoms (LUTS). Hormone replacement therapy (HRT) has been the primary choice for addressing these problems. However, long-term prescription of HRT can result in several adverse effects, including a higher risk of breast cancer and cardiovascular diseases. Phytoestrogen, an estrogen-like compound derived from plants such as purple sweet potatoes (*Ipomoea batatas* L.), contains anthocyanin that could serve as a better alternative to estrogen replacement therapy. This study aimed to demonstrate the effects of phytoestrogens in purple sweet potatoes on the histomorphology of the bladder in menopausal female Wistar rats (*Rattus norvegicus*). This experimental study used a posttest-only control group design. A total of 36 Wistar rats undergoing ovariectomy were randomly assigned into two groups, with 18 samples in each group. The experimental group received the purple sweet potato ethanol extract orally, while the control group received a placebo. The structures of the smooth muscle and urothelial layer of the bladder were observed using a microscope. Data were analyzed using an independent t-test to compare bladder smooth muscle and urothelial layer thickness between groups, with a significance of $p<0.05$. A normality test was performed to determine the normal distribution of the data. Normally distributed data were assessed to find the mean and standard deviation (SD). Significant differences were found in the mean thickness of the smooth muscle and urothelial layer between both groups ($p=0.00$), with both being thicker in the experimental group. In conclusion, phytoestrogens in purple sweet potatoes can influence the histomorphology of the bladder. Ovariectomized female Wistar rats that received purple sweet potato ethanol extract exhibited thicker smooth muscle and urothelial layer of the bladder.

Keywords: Purple sweet potatoes; smooth muscle thickness; postmenopause; lower urinary tract symptoms; human & health

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Highlights:
1. Purple sweet potatoes cultivated in Bali, Indonesia, were found to be rich in phytoestrogen due to the high levels of anthocyanin-type flavonoids.
2. The phytoestrogen in purple sweet potato ethanol extract demonstrated estrogenic activity and the potential to substitute hormone replacement therapy (HRT) as the treatment of lower urinary tract symptoms.

INTRODUCTION

Menopause is one of the changes that older women experience due to the inability of ovarian follicles to produce estrogen and inhibin, resulting in various changes in organ structure and function as well as an increased risk of developing chronic diseases. One of the hypoestrogenism symptoms during menopause that greatly interferes with quality of life is lower urinary tract symptoms (LUTS), which includes stress or urge urinary incontinence, nocturia, a weak urine stream, straining, urinary hesitancy, and terminal dribbling, among others (Azadzoi & Siroky 2013, Varella et al. 2016). A decreasing estrogen level in postmenopausal women will cause cell apoptosis, which leads to atrophy of the smooth muscle and urothelial layer in the bladder. Atrophy of smooth muscle will reduce the contractility of the bladder and also reduce the thickness of the urothelial layer (Birder et al. 2012, Robinson et al. 2013).

Since all of the clinical symptoms are caused by a decrease in estrogen levels, the most rational treatment is estrogen-based hormone replacement therapy (HRT). Several studies have shown that HRT effectively eases lower urinary tract symptoms in menopausal women (Lobo et al. 2014). However, even though HRT provides excellent benefits, more than 80% of women who have received it do not intend to continue the treatment due to various medical reasons. Side effects of HRT include an increased risk of blood clots, liver disease, and cancer. Since HRT carries several potential complications, it is necessary to search for an estrogen substitute (Bedell et al. 2014).

A number of plants produce naturally-sourced compounds that may serve as a substitute for estrogen. One of the compounds is phytoestrogen, which has estrogen-like effects without the harmful side effects of estrogen (Desmawati & Sulastri 2019). Phytoestrogens are divided into four types: isoflavones, flavonoids, stilbenes, and lignans. Because of their similar molecular structure to estrogen, flavonoid compounds can bind to and activate estrogen receptors on target cells. Several phytoestrogens have been found to bind to both types of estrogen receptors, i.e., estrogen receptor beta (ERβ) and estrogen receptor alpha (ERα). The binding of phytoestrogens to receptors can induce the expression of estrogen-responsive genes and trigger cell proliferation (Sugiritama 2020).

Purple sweet potatoes cultivated in Bali, Indonesia, are potentially useful as a source of phytoestrogens due to their high content of anthocyanin-type flavonoids. The results of a preliminary study revealed that purple sweet potato ethanol extract given to menopausal animal models was able to increase ERα and ERβ messenger ribonucleic acid (mRNA) expression at an optimal dose of 4 mL per day. Compared to the control group, female rats that underwent bilateral ovariectomy and received purple sweet potato ethanol extract had thicker vaginal epithelium and a better degree of maturation (Yuwono et al. 2018, Adnyana et al. 2019). The purpose of this study was to prove the effects of purple sweet potato ethanol extract on the bladder histomorphology of menopausal animal models. The effects were analyzed by measuring the thickness of the smooth muscle and urothelial layer of the bladder.

MATERIALS AND METHODS

This was an experimental study with a posttest-only control group design. This study was conducted in the Histology Laboratory, Faculty of Medicine, Universitas Udayana, Denpasar, Indonesia, between April and November 2022. We received ethical approval from the Ethics Committee of the Faculty of Medicine, Universitas Udayana (No. 182/UN14.2.2.VII.14/LT/2022 on 2/1/2022). This study utilized healthy female Wistar rats (Rattus norvegicus). The number of samples was determined by the Federer formula (Dharmawati et al. 2019). According to the formula, 36 rats weighing 200–250 g, aged 10–12 weeks, and undergoing ovariectomy were used as samples.

Ovariectomy (OVX), or the removal of the ovaries, started with the induction of anesthesia. Isoflurane with a 5% concentration was used for the induction, and it was maintained at a 2.5% concentration. Coaxial nose cones were used to deliver oxygen. We performed bilateral ovariectomy using a double dorsolateral approach (Sophocleous & Idris 2019, Souza et al. 2019). The rats were anesthetized and fixed with plaster. The rats' dorsal ridges were shaved bilaterally. As the ovaries were located on both sides of the abdomen and below the kidneys, we incised the skin medially to the most prominent dorsal region. In young or thin rats, a bulge that could indicate the incision site might not be visible. Therefore, the incision site was found by placing a thumb on the uppermost proximal thigh. The base of the distal phalanx was incised medially. In order to show the dorsolateral abdominal muscles, such as the external oblique muscle, a 1.5 cm area of the skin was incised. By dissecting the muscle, the adipose tissue surrounding the ovary was revealed. As a result, it created an entrance to the peritoneal cavity (Souza et al. 2019, Setiawan et al. 2022). In order to prevent the detachment of small ovarian fragments, fat around the incision site was carefully removed. After identifying the ovary and uterine horn, the ovarian tissue was removed in a single step by ligating the distal horn. The rats' uterine horns,

muscles, and skins were sutured at the end of the procedure (Khajuria et al. 2012).

A total of 36 rats were randomly divided into two groups of 18 rats. The experimental group received 4 mL of purple sweet potato ethanol extract orally per day, while the control group received a saline solution. The purple sweet potato ethanol extract contained 119 mg/mL of anthocyanins (Setiawan et al. 2022). Rats that died or became ill were dropped out of the experiment.

Locally grown purple sweet potatoes (*Ipomoea batatas* L.) were used as the source material for the ethanol extract in this study. After peeling the skin, the sweet potatoes were transversely cut into thick slices of 2–2.5 cm. One kg of sweet potatoes was soaked in 1 L of 90% liquid before being filtered through three layers of gauze. The next step was boiling the filtered liquid (Sugiritama 2020). This material contained 119 mg/mL of anthocyanin. After successfully producing the ethanol extract, 1 mL of distilled water was added to the extract for each rat. Spectrophotometer measurements indicated anthocyanin absorption at 520 and 720 nm. The fresh purple sweet potatoes had significant quantities of anthocyanin.

All post-ovariectomy procedures lasted 30 days. The rats were terminated at the conclusion of the experiment. Histological preparations were made from a 1×1 cm segment of the rats' bladder and stained with hematoxylin and eosin. The bladder's smooth muscle and urothelial layer thicknesses were measured using an Olympus CX41 microscope (Olympus Corp., Japan) and an Optilab camera (Optilab, Indonesia) with 400X magnifying power. The measurement points were from the base to the top of the smooth muscle and urothelial layer (Zderic & Chacko 2012). Measurements were expressed in micrometers (µm).

Data obtained from the experiment were analyzed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, N.Y., USA). We conducted a normality test to determine the normal distribution of the data. Numerical data with a normal distribution were provided as mean±standard deviation (SD). We also conducted an independent t-test to compare the thickness of the bladder smooth muscle and urothelial layer between the two groups. A p-value of <0.05 was considered significant (Hazra & Gogtay 2016).

RESULTS

A normality test was performed to analyze the data. All variables showed a normal distribution, as indicated by the mean and standard deviation. The results of the analysis are shown in Table 1.

Figure 1 shows the smooth muscles as well as the urothelial layers of both the control group and the experimental group. When compared to the control group, the smooth muscles and urothelial layers were found to be significantly thicker in the experimental group.

Table 1. Normality test results of the control and experimental groups.

Variables	Mean (µm)	SD	Normality
Urothelial layer thickness			
Control group	7.78	7.62	0.13
Experimental group	40.56	17.88	0.53
Smooth muscle thickness			
Control group	27.08	12.93	0.53
Experimental group	48.13	17.45	0.65

Notes: SD=Standard deviation

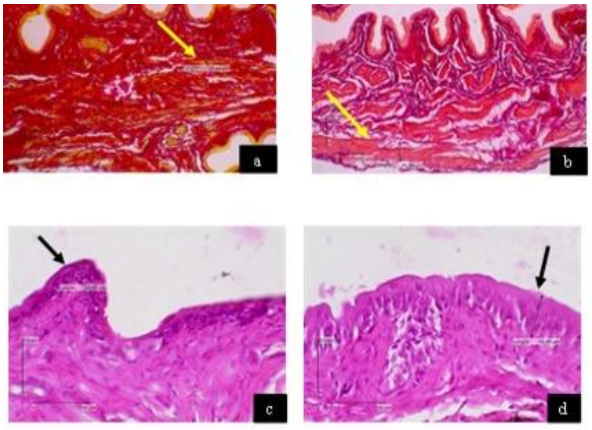

Figure 1. Histological picture of the result of the study using a microscope with 400x magnification. (a)Smooth muscle of the control group; (b)Smooth muscle of the experimental group; (c)Urothelial layer of the control group; (d)Urothelial layer of the experimental group.

An analysis using an independent t-test was performed to determine the statistical significance of the differences in smooth muscle and urothelial layer variables between the control and experimental groups. The results revealed that there was a statistically significant difference in the

bladder smooth muscle and urothelial layer thickness between the two groups ($p<0.05$). The p-values for both variables were 0.00, indicating statistically significant differences in smooth muscle and urothelial layer thickness between the control group and the experimental group (Table 2).

Table 2. Independent t-test results between the control and experimental groups.

Variables	95% CI	p
Urothelial layer thickness		
Control group Experimental group	-29.67 – (-10.72)	0.00
Smooth muscle thickness		
Control group Experimental group	-31.45 – (-10.65)	0.00

Notes: CI=Confidence interval

The results of the analysis indicated that the experimental group had significantly thicker smooth muscle and urothelial layer compared to the control group. These findings suggested that the administration of purple sweet potato ethanol extract had effects on the thickness of these layers.

DISCUSSION

Our experiment aimed to determine the effects of phytoestrogens on the histomorphology profile of ovariectomized rats' bladders. The experiment was focused on the smooth muscle and urothelial layer of the bladder. Ovariectomized rats have been used as an established menopausal model in research (Rejeki et al. 2018). The female lower urinary tract has been reported as a target organ for the action of sex steroid hormones. The bladder urothelium and smooth muscle are sensitive to changes in circulating estrogen, so ovariectomy can induce significant urothelial and smooth muscle atrophy (Liang et al. 2013). In an observation two weeks following ovariectomy, the amount of circulating estradiol decreased by 50%. The estradiol concentration remained relatively constant for six weeks after the ovariectomy (Malone et al. 2014). In this study, the optimal time to begin intervention was determined to be two weeks after ovariectomy.

The effects of phytoestrogen on the menopausal bladder were still unclear. Our study provides valuable information on the histological measurement of smooth muscle and urothelial layer thickness after the administration of phytoestrogen (Figure 1). Yuwono et al. (2018) assessed the effects of phytoestrogen on the female genitalia of menopausal rats. They found that intervention using purple sweet potato ethanol extract enhanced epithelial maturation and differentiation and increased epithelial thickness in rats that underwent oophorectomy. In this current study, we found significant differences in the bladder urothelial layer and smooth muscle thickness between the two groups, with the experimental group having a higher mean thickness.

In this study, the thickness of bladder layers increased despite phytoestrogen being a non-steroidal polyphenolic compound derived from plants. Phytoestrogen can carry out the same biological function as estrogen. It has been suggested that purple sweet potato ethanol extract contains phytoestrogen compounds and has potential estrogenic activity. Orally administered purple sweet potato ethanol extract undergoes intestinal metabolism to be converted into an active substance by the normal intestinal flora (Sugiritama et al. 2022). This compound resembles estrogen structurally, though its estrogenic activity is slightly lighter.

Phytoestrogen enters the bloodstream and binds to estrogen receptors in the bladder cells. In the present study, phytoestrogens were found to bind to urothelial cells and smooth muscle. The phytoestrogen content selectively modulates the estrogen receptor. After binding to the estrogen receptor, it modulates transcription and gene expression to induce changes in cell physiology. This will trigger the proliferation of cells and increase the thickness of the cell layer (Tao et al. 2022). Smooth muscle cell proliferation and migration are crucial events in the pathophysiology of vascular diseases. In ovariectomized mice, the aortic tissue sections stained with hematoxylin and eosin exhibited increased wall thickness and vascular smooth muscle hyperplasia (Yuwono et al. 2018, Sugiritama et al. 2022).

Several studies have suggested that the thickening of the bladder smooth muscle can increase its contractility. There is a strong correlation between smooth muscle contractility and urethral resistance, both of which play an essential role in the mechanism of lower urinary tract symptoms. When distention of the bladder wall occurs, the smooth muscle stimulates the spinal cord and pons for sympathetic inhibition, somatic nerve inhibition, and parasympathetic stimulation (Zderic & Chacko 2012). The voiding phase begins when the rhabdosphincter and bladder neck relax while the smooth muscle contracts and urethral resistance decreases, allowing urine to be expelled. Smooth muscle contraction is a factor that plays a vital role in the micturition mechanism. If the contraction is inadequate, the micturition process will be disrupted, resulting in lower urinary tract symptoms, especially voiding symptoms. By increasing the thickness of the smooth muscle layer, it is

anticipated that the lower urinary tract symptoms will become less severe (Aikawa et al. 2003, Valentini et al. 2016).

Urothelium consists of cells with a variety of functions. It separates the contents of the bladder from the underlying lining. It also functions as a sensory organ that conveys chemical and physical stimulation to the afferent nervous system and underlying smooth muscle (Birder et al. 2012). Changes in the urothelium can influence the growth and function of smooth muscle cells and afferent nerves, resulting in lower urinary tract symptoms. Numerous studies have demonstrated that a thin urothelial layer is a risk factor for lower urinary tract symptoms, including overactive bladder and bladder pain syndrome (interstitial cystitis) (Birder & Andersson 2013).

Reduced permeability caused by a thin urothelium can increase the likelihood of water, urea, and other harmful substances being present in the urine and infiltrating the underlying tissue, including tissues in the nerves. This will increase the incidence of bladder inflammation and cause lower urinary tract symptoms (Dalghi et al. 2020). As the urothelial layer normally responds to stimuli from the outside of the cell, the thinning of the urothelial layer diminishes sensory function. When the urothelium is stimulated, it releases molecular and cellular mechanisms that enable it to detect the stimulus. This results in the modulation of smooth muscle contraction and relaxation, which is crucial to the micturition mechanism. The urothelium releases various mediators, such as adenosine triphosphate (ATP), acetylcholine, prostaglandins, nitric oxide, and nerve growth factor (Birder et al. 2012, Keay et al. 2014, Sellers et al. 2018). It is anticipated that by increasing the thickness of the bladder smooth muscle and urothelial layer, lower urinary tract symptoms will improve. Further research is required to support the evidence that increasing the thickness of both the smooth muscle and urothelial layer can alleviate or prevent lower urinary tract symptoms in menopausal women.

Strength and limitations

This study can demonstrate the potential of purple sweet potato ethanol extract as a natural source of phytoestrogen. Therefore, it can be used as an alternative to hormone replacement therapy (HRT) for treating lower urinary tract symptoms. The phytoestrogen in purple sweet potato ethanol extract has the ability to increase smooth muscle and urothelial layer thickness. However, due to the short duration of this study, it might not be able to demonstrate the long-term effects or potential side effects associated with prolonged use of purple sweet potato ethanol extract.

CONCLUSION

This study provides strong evidence that phytoestrogens in purple sweet potatoes can influence the histomorphology of the bladder. The intervention of orally administering the purple sweet potato ethanol extract resulted in increased smooth muscle and urothelial layer thickness in the experimental group compared to the control group of menopausal female rats.

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Conflict of interest

None.

Ethical consideration

This study received ethical approval from the Ethics Committee of the Faculty of Medicine, Universitas Udayana, Denpasar, Indonesia, with registration No. 182/UN14.2.2.VII.14/LT/2022 on 2/1/2022.

Funding disclosure

None.

Author contribution

BAP contributed to the conceptualization and study design. BAP, KBS, and IWN contributed to the methodology, data curation, investigation, and original draft preparation. NG, GWKD, and IBMS contributed to the investigation, original draft preparation, and supervision.

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Case Series

MATERNAL DEATHS CAUSED BY COVID-19 INFECTION IN THE FIRST YEAR OF THE PANDEMIC WAVE

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ABSTRACT

This article presents seven cases of maternal deaths attributed to COVID-19 during the first year of the pandemic wave. These cases provide insights into the natural progression of COVID-19 in pregnant women who were not vaccinated. This study showed that COVID-19 significantly increased maternal and neonatal mortality and morbidity. All of the patients exhibited symptoms of fever, cough, and dyspnea upon admission to the hospital. They were admitted with elevated respiratory rates (26–32 times/minute) and low oxygen saturation (<95%). Four patients had obesity, while one patient had pregestational diabetes. The COVID-19 diagnosis was established using a rapid antibody or antigen test and chest X-ray, which indicated pneumonia. Medical interventions administered to the patients included antiviral therapy (5 patients), antibiotics (6 patients), and anticoagulants (4 patients). From a total of five babies delivered, four babies were delivered via cesarean section. Two babies were not delivered due to previability and maternal deaths before delivery. The patients passed away within 3–10 days of hospital admission. In conclusion, adequate and early intervention and management of pregnant women infected with COVID-19 are crucial in preventing maternal and neonatal deaths, especially in unvaccinated women.

Keywords: COVID-19; maternal mortality; maternal health; pregnancy

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Highlights:
1. These cases of maternal deaths caused by COVID-19 infections illustrated the significant risk factors for maternal mortality during the early phases of the pandemic, while studies had not extensively reported this.
2. COVID-19 infections increase the risk of maternal and neonatal mortality, with infants having a lower chance of survival even if they are delivered.
3. Respiratory support, antiviral medications, antibiotics, anticoagulants, and supportive care are the primary treatments for severe COVID-19 in pregnancy.

INTRODUCTION

The COVID-19 pandemic has lasted for several years and created a huge health impact around the world. COVID-19 had infected a total of 109,167,919 people worldwide as of February 2021, with a mortality rate of 2,406,669 people (Worldometer 2021). Pregnant women become a high-risk population due to the anatomic, physiologic, hormonal, and immunologic changes during pregnancy. Anatomic and physiologic changes in the lung and chest during pregnancy

decrease maternal tolerance to hypoxia. Changes in the cell-mediated immune response during pregnancy will also increase the susceptibility of pregnant women to viral infection (Zaigham & Andersson 2020). Previous studies showed that COVID-19 increased morbidity during pregnancy, but the mortality rate was still quite low. In a meta-analysis study by Allotey et al. (2020), COVID-19 increased the risk of adverse pregnancy outcomes, including preterm delivery, preterm premature rupture of the membrane (PPROM), a higher cesarean section rate, hemorrhage postpartum, intrauterine fetal death (IUFD), neonatal death, and an abnormal Apgar score. Conditions such as postpartum hemorrhage and unviable gestational age can be direct and indirect causes of death in pregnant women. The percentage of maternal mortality caused by COVID-19 in a previous study was 0.1%, with 76 mortality cases in 11,580 pregnancies (Allotey et al. 2020, Sulistyono et al. 2020). A study by Zaigham & Andersson (2020) found no maternal deaths in a total of 108 pregnancy cases with COVID-19. However, we found from our study that COVID-19 significantly increased the risk of maternal death by eightfold. Vaccination status is a crucial prognostic indicator for COVID-19-infected maternal deaths. In our previous study, all of the mothers were unvaccinated against COVID-19 because the government had not yet implemented vaccination programs at the time (Akbar 2021, Akbar et al. 2022).

Until early 2021, Indonesia had become one of the Southeast Asian countries with the fastest-developing COVID-19 cases. The official data from the Indonesian Ministry of Health revealed that the total number of cases of COVID-19 was 1,210,000, with a mortality rate of 32,936 people. The percentage of the fatality rate was 3.12%. The average daily number of new cases in Indonesia was 10,000 cases per day in early 2021 (Satuan Tugas Penanganan COVID-19 2020). Rumah Sakit Universitas Airlangga is a teaching hospital that has become a referral center for maternal COVID-19 in Surabaya, Indonesia. Until mid-2021, Rumah Sakit Universitas Airlangga had delivered more than 300 cases of pregnancy with suspected COVID-19. In this case series, we present seven maternal deaths directly caused by COVID-19 at Rumah Sakit Universitas Airlangga.

CASE SERIES

We chose seven cases of maternal deaths caused by COVID-19 infection during pregnancy in the first year of the pandemic. During this early phase of the pandemic, no mothers had received the COVID-19 vaccine because the government had not yet implemented the program. All patients presented with a fever, cough, and dyspnea. The patients upon admission had increased respiratory rates (between 26 and 36) and low oxygen saturation (95%), which were indicative of dyspnea. Rapid antibody tests (n=6), rapid antigen tests (n=2), and chest X-rays (n=7) were utilized to diagnose all patients. The patients were treated with pharmacological and non-pharmacological treatments, as well as obstetric care. As a medical intervention, the patients received antiviral therapy (n=5), antibiotics (n=6), and anticoagulants (n=4). Five infants were delivered, four of them by cesarean section. However, two infants were not delivered due to previability (less than 24 weeks), and maternal and fetal mortality occurred prior to delivery. Only three infants survived and were then discharged from the hospital. The three others were stillbirths, and one infant passed away an hour after birth. One patient experienced postpartum hemorrhage following a cesarean section due to uterine atony and required a hysterectomy. The length of stay in these cases ranged from four to ten days.

Case 1

A 38-year-old woman, Mrs. J, was a multiparous patient with a gestational age of 33–34 weeks. She was referred to the hospital with complaints of fever in the last two weeks, coughing, and shortness of breath for a week. The current pregnancy was her second, with a previous history of sectio caesarea (SC) 10 years ago. During the physical examination, we found that the oxygen saturation was 60%, with a fast respiration rate of 32 breaths per minute. The results of COVID-19 screening showed positive immunoglobulin M (IgM) antibodies and an increased neutrophil-to-lymphocyte ratio (NLR), while the chest radiograph showed bilateral pneumonia. The patient also had a comorbidity of class II obesity. We treated the patient with 15 L/min oxygen supplementation using a non-rebreathing mask and medicines, i.e., oseltamivir, levofloxacin, and other supportive therapy. The next day, the patient showed signs of respiratory failure (oxygen saturation of 86.3%), so we decided to put her on a ventilator and perform an emergency SC to improve her condition. A baby girl was born with a 1,960-g body weight, 46-cm body length, and an Apgar score of 7–8. After the surgery, the mother's condition continued to deteriorate. Two days postoperatively, the patient showed worsened respiratory failure (70% saturation) and decreased consciousness, with a Glasgow Coma Scale (GCS) score of 111. We performed chest compression for five cycles, but it was unsuccessful, and the patient died.

Case 2

A 28-year-old woman, Mrs. LH, was 21–22 weeks pregnant and referred to the hospital with complaints

of coughing and shortness of breath for three days. At the previous hospital, the patient had symptoms of impending respiratory failure, with a respiration rate of 32 breaths per minute and 77% oxygen saturation. Previously, the anesthesiologist performed intubation, put the patient on a ventilator, and referred the patient to our hospital because the intensive care unit (ICU) was fully occupied. On arrival, the patient was already on a ventilator with a positive end-expiratory pressure (PEEP) of 8, a 100% fraction of inspired oxygen (FiO2), and a 95% oxygen saturation. This was the patient's second pregnancy, with a previous history of SC in the previous five years. The patient had a comorbidity of class I obesity. The laboratory examination results revealed an increased C-reactive protein (CRP) level, anemia, hypoalbuminemia, hypokalemia, and acidosis (pH=7.33). The chest radiograph indicated bilateral pneumonia. We diagnosed the patient with respiratory failure and treated her with oxygen supplementation using a ventilator, moxifloxacin, azithromycin, and other supportive drugs. During the insertion of the central venous catheter (CPC) on the third day of treatment, an iatrogenic pneumothorax occurred, necessitating the use of water seal drainage (WSD). On the fifth day, the patient experienced rapid deterioration, with 75% oxygen saturation while using the ventilator, a PEEP of 11, and a FiO2 of 100%. Respiratory failure occurred, and the patient eventually died. The delivery did not happen because, according to the evaluation, the baby was not yet viable. Later, it was announced that the cause of death was acute respiratory distress syndrome due to COVID-19 pneumonia.

Case 3

A 32-year-old woman, Mrs. H, was 30–31 weeks pregnant. It was her fifth pregnancy, with a history of three abortions. The patient came to the hospital complaining of coughing and shortness of breath for three days. Previously, the patient received antenatal care twice: once with obstetricians and twice at the primary health center. She was categorized as having a high-risk pregnancy because of her history of recurrent miscarriage, class II obesity, and pre-pregnancy diabetes. On arrival at our hospital, the patient had shortness of breath. The respiration rate was 30 breaths per minute while using a 6 L/min oxygen mask, and the oxygen saturation was 95%. The laboratory examination results showed an increased random blood sugar level (216 mg/dL) and CRP, while the chest radiograph showed bilateral pneumonia. In accordance with the gestational age (30 weeks) and the estimated fetal weight (1,500 g), conservative obstetric treatment was administered. The medical management included the administration of medications, i.e., oseltamivir and azithromycin, as well as injections of levemir and novorapid for blood sugar regulation and other supportive therapy. On the second day of treatment, the patient's condition worsened, with a decreased oxygen saturation of 89%. Therefore, the patient was put on a ventilator and admitted to the ICU. On the fourth day of treatment, when the mother had respiratory failure and metabolic acidosis, we decided to perform an emergency termination for maternal-fetal lifesaving. A baby boy was born through SC with a 1,615-g body weight, 36.5-cm body length, and an Apgar score of 1–1–1. Unfortunately, the baby died two hours after birth. On the seventh day of treatment, widespread pneumonia was seen on the chest radiograph. In addition, a blood culture showed infection with *Staphylococcus hominis*, so additional meropenem antibiotic therapy was administered. In the ICU, the antiviral therapy was changed to remdesivir with a dose of 200 mg daily. On the tenth day of treatment, the patient's condition continued to worsen, and further respiratory failure occurred. The oxygen saturation was 70% while using the ventilator. Later that day, the patient died. The cause of death was acute respiratory distress syndrome due to COVID-19 pneumonia, with comorbid obesity and pregestational diabetes mellitus.

Case 4

Mrs. NA, 31 years old, was 27–28 weeks pregnant and referred to the hospital with complaints of coughing, cold, and fever for two days. This was her second pregnancy with a history of SC at 27 weeks gestation. On arrival, the patient had shortness of breath with a respiration rate of 26 breaths per minute and an oxygen saturation of 98% while using an 8 L/min oxygen mask. The COVID-19 screening showed a positive antigen swab and reactive immunoglobulin G (IgG), while the chest radiograph showed bilateral pneumonia. The patient was treated using 10 L/min oxygen therapy, a non-rebreathing mask, oseltamivir, and other supportive therapy. We decided to maintain the pregnancy with conservative care according to the gestational age. On the fourth day of treatment, the condition worsened. The patient experienced respiratory failure with an oxygen saturation of 93%; thus, the patient was put on a ventilator and admitted to the ICU. During the treatment, we found decreased levels of albumin, potassium, and hemoglobin, as well as increased levels of ferritin. While using the ventilator, the patient's oxygen saturation remained low (93–95%), and intrauterine fetal death (IUFD) occurred. On the seventh day of treatment, the oxygen saturation decreased to 70%. The condition continued to worsen until the patient died. The cause of death was acute respiratory distress syndrome due to COVID-19 pneumonia.

Case 5

A 41-year-old woman, Mrs. S, was 39 weeks pregnant. This was her second pregnancy following a cesarean delivery seven years prior. The patient was referred to the hospital with complaints of coughing, spasms, a headache, nausea, and vomiting. On arrival, the patient had shortness of breath. The respiration rate was 36 breaths per minute while using a non-rebreathing mask on 10 L/min oxygen supplementation. The oxygen saturation was 55–60%. We found a blood pressure of 189/110 mmHg and a urine protein of +3. Thus, we diagnosed the patient with severe pre-eclampsia. According to the results of COVID-19 screening, we found that the IgG was reactive with bilateral pneumonia. For the initial management, we administered magnesium sulfate (MgSO4) injections, nifedipine, and methyldopa for the severe pre-eclampsia condition. In accordance with the gestational age, severe pre-eclampsia diagnosis, location of the breech, and additional COVID-19 pneumonia, we decided to perform SC for the delivery. A baby boy was born with a 2,655-g body weight, a 49-cm body length, and an Apgar score of 3-5. Uterine atony occurred during the SC procedure, and uterine compression sutures were performed unsuccessfully using the Surabaya Method (Jiang et al. 2020). Later on, a supravaginal hysterectomy (SVH) was performed. Postoperatively, the patient was admitted to the ICU and put on a PEEP of 10 on the ventilator, with an oxygen saturation of 95–99% and hemoglobin of 8.1 mg/dL. During treatment, we found that the levels of procalcitonin, D-dimer, hemoglobin, and albumin decreased. The patient received daily doses of 750 mg levofloxacin therapy and 100 mg cortisone, as well as 300 units/hour of heparin, 300 mg chloroquin twice a day, and other supportive therapy. In the ICU, the patient's condition worsened, and the oxygen saturation was unstable (80–93%). On the fifth day of treatment, the patient died. The cause of death was acute respiratory distress syndrome due to COVID-19 pneumonia with complications of severe pre-eclampsia, postpartum hemorrhage due to uterine atony, and class I comorbid obesity.

Case 6

Mrs. N, 31 years old, was referred from a rural hospital (193 km from our hospital) with chief complaints of shortness of breath. This was her second pregnancy, with a gestational age of 27–28 weeks and an estimated fetal weight of 900 g. The patient experienced coughing, a cold, and joint pain for two weeks prior to being admitted to the previous hospital. The patient was a physician and already had supportive medicines at home before coming to the previous hospital. After five days of treatment, the fever and cough worsened, and anosmia symptoms started to occur. After a week of treatment at the previous hospital, a swab was collected for a polymerase chain reaction (PCR) test, and the result was COVID-19 positive. Afterwards, the patient was referred to our hospital for further treatment. On arrival at our hospital, the patient had shortness of breath with a respiration rate of 32 breaths per minute and 91% oxygen saturation. The laboratory results revealed mild anemia, hypoalbuminemia, and elevated NLR, CRP, and D-dimer levels. The patient was then given oxygen supplementation through a high-flow nasal cannula (HFNC), daily doses of 500 mg azithromycin and 750 mg levofloxacin, twice-daily doses of 75 mg oseltamivir and 6 mg dexamethasone injections (for fetal lung maturation), and other supportive therapy. Because of the worsening symptoms, we decided to put the patient on a ventilator with a PEEP of 10 and 100% FiO2. On the fourth day of treatment, when vaginal bleeding occurred, we performed pregnancy termination by administering 100 mg of misoprostol vaginally every six hours. A baby girl was born, weighing 1,000 g and measuring 39 cm in length, with an Apgar score of 0. We also found a high interleukin-6 (IL-6) level, necessitating a 600 mg Actrema injection. During hospitalization, the symptoms worsened with a gradual decrease in oxygen saturation. On the sixth day of treatment, the patient's condition worsened with 53% oxygen saturation and blood pressure. The pulse continued to decrease despite dopamine administration. Later that day, the patient died after chest compressions were performed. The cause of death was acute respiratory distress syndrome (ARDS) due to COVID-19 pneumonia.

Case 7

Mrs. K, 31 years old, was referred to a private hospital after experiencing shortness of breath for two days, along with coughing and fever for three days. This was her first pregnancy, and she was 35 weeks pregnant without any additional complications or comorbidities. On arrival, the patient had shortness of breath with a respiration rate of 26–28 breaths per minute and 91% oxygen saturation. The blood pressure was 130/70 mmHg, and the pulse was 120 beats per minute. We found bilateral pneumonia on the chest radiograph, with 16,900 leukocytes and increased liver function, as well as elevated D-dimer (6.2 ng/mL), CRP (219.4 mg/L), and NLR (23.25) levels. The patient was given oxygen supplementation, twice-daily doses of 75 mg oseltamivir and 300 mg chloroquin, 400 units of heparin per hour, and other supportive therapy. We decided that the pregnancy would be terminated after lung maturation with twice-daily doses of 6 mg dexamethasone via intramuscular (IM) for two days. On the second day of treatment, fetal bradycardia

occurred. An emergency SC was then performed, and a baby girl was born with 2335 g of body weight, 44 cm of body length, and an Apgar score of 3–5–7. Two days after surgery, the patient's oxygen saturation dropped to 88%, so we decided to put her on a ventilator. However, the patient's condition continued to worsen. On the seventh day of treatment, the patient died. The cause of death was acute respiratory distress syndrome due to COVID-19 pneumonia.

DISCUSSION

Since March 2020 until the end of January 2021, Rumah Sakit Universitas Airlangga had delivered 108 babies related to confirmed COVID-19 infections. However, 81 highly suspect cases of COVID-19 were unable to be confirmed using reverse transcription polymerase chain reaction (RT-PCR) due to a lack of available supplies. There were seven maternal deaths from 108 cases related to COVID-19, with a case fatality rate of 6.48%. All the maternal deaths were caused by acute respiratory distress syndrome (ARDS) related to COVID-19. The maternal background, clinical symptoms, and diagnosis of the patients in this case series can be seen in Table 1. The maternal age range was 28–41 years old. Six patients were multiparous, with gestational ages of 22–40 weeks during admission. Three patients came to our hospital by themselves, while the rest were referred by other hospitals because of worsening conditions during treatment (Dewantiningrum et al. 2023).

Table 1. Maternal background, clinical symptoms, and diagnosis of the pregnant women.

	J	LH	H	NA	S	N	K
Age (years)	38	28	32	31	41	31	31
Length of stay (days)	4	5	10	8	6	8	8
ANC	Hospital 9x	Mother and Child Hospital 5x	PHC 2x, Specialists 2x	Hospital 5x	PHC 4x, Hospital 1x	Hospital 4x	PHC 5x
Referral	Referred by the previous hospital	Referred by the previous hospital	Came by herself	Came by herself	Came by herself	Referred by an out-of-town hospital	Referred by the previous hospital
Gestational age at admission (weeks)	33	22	30	27	40	27	35
Comorbidity	Class I obesity	Class I obesity	Class I obesity and pregestational diabetes mellitus	-	Class I obesity	-	-
Symptom	Fever, dyspnea, and coughing (1-2 weeks)	Coughing and dyspnea (3 days)	Coughing, dyspnea, and fever (3 days)	Fever, coughing, and flu-like symptoms (2 days)	Dyspnea, headache, and nausea	Dyspnea	Coughing, myalgia, fever, and dyspnea (4 days)
Blood pressure (mmHg)	101/68	128/64	117/63	113/74	189/110	109/61	130/70
Respiratory rate (breaths/minute)	32	32	30	26	36	32	28
Temperature (ºC)	36.7	37.7	36	36.5	36.4	36.8	37.8
Oxygen saturation during admission (%)	60% in a free O_2 room, 96% with 15 L/min of O_2 using NRM	77% in a free O_2 room, 90% with 12 L/min of O_2 using NRM	95% with 6 L/min of O_2 using a simple mask	98-99% with 8 L/min of O_2 using a simple mask	55-60% with 10 L/min of O_2 using NRM	90% with 10 L/min of O_2 using NRM	92% with 10 L/min of O_2 using NRM
Rapid antibody test	(+)	N/A	(+)	(+)	(+)	(+)	(+)
Rapid antigen test	N/A	N/A	N/A	(+)	N/A	N/A	(+)
Chest X-ray	Bilateral pneumonia	Bilateral pneumonia	Bilateral pneumonia	Bilateral pneumonia	Bilateral pneumonia	Bilateral pneumonia	Bilateral pneumonia

PHC: Primary health center. NRM: Non-rebreathing mask. N/A: Not available.

Six patients came with the chief complaint of dyspnea, a respiratory rate of 26–36 breaths/minute, and oxygen saturation in free roam between 50–95%. There were only two patients with fevers (t>37.5°C) during admission. Symptoms of shortness of breath and fever indicate the presence of COVID-19 pneumonia, which seems to be an indicator that COVID-19 will be severe or critical. According to a study, fever (40%), cough (39%), and shortness of breath (19%) were the most common clinical manifestations seen in pregnant women with COVID-19 infection. However, the likelihood of fever in pregnant women is lower than in non-pregnant women infected with COVID-19 (OR=0.43) (Allotey et al. 2020, Villar et al. 2021; Atmaja et al. 2022). In this case series, all patients experienced symptoms within 2–4 days. As for Mrs. J, the patient had the symptoms for 1–2 weeks, which might indicate a delay in the COVID-19 diagnosis, treatment, and therapy (Table 1). According to our analysis, the root cause might be that all cases in this study happened at the beginning of the pandemic, thus indicating that neither the patients nor medical personnel had a high suspicion of COVID-19.

Six patients had positive results from the rapid antibody tests, and all cases had bilateral pneumonia on chest X-ray imaging. At the beginning of the pandemic, we used serological antibody tests to screen for COVID-19, given the limitations of PCR testing at our center. A combination of clinical symptoms, laboratory tests, chest X-rays, and rapid antibody tests may detect the majority of COVID-19 cases at our hospital (Laksana et al. 2020, Wardhana et al. 2021, Akbar et al. 2023). However, due to its limited sensitivity and specificity, the serologic antibody test as a screening method has now been replaced by the antigen swab test. The presence of pneumonia is considered a more sensitive indicator of COVID-19, as the sensitivity of a chest CT scan may exceed that of an RT-PCR swab examination in the early phase of infection (Rashid et al. 2020, Porte et al. 2020, Mair et al. 2021).

The clinical management and pregnancy outcomes are summarized in Table 2. The patients were treated mainly with antivirals, antibiotics, and anticoagulants based on their personal conditions, in addition to supportive therapy. According to the COVID-19 guidelines, therapies that should be given were still very diverse, and the management of COVID-19 in pregnancy at our hospital also varied depending on the patient's condition (Laksana et al. 2020). In this study, five patients received oseltamivir as their antiviral therapy. Oseltamivir is a neuraminidase inhibitor that is widely used for influenza A and B therapy. The structure of the spike S1 protein in SARS-CoV-2 resembles that of neuraminidase. Therefore, neuraminidase inhibitor drugs, such as oseltamivir, can be useful in SARS-CoV-2 therapy. In silico, in vitro, and clinical studies by Tan et al. (2020) in Wuhan, China, showed that oseltamivir was ineffective in the therapy of COVID-19. From the total of 79 COVID-19 patients that used oseltamivir, remission was found in only 22.58% of the patients, a number much smaller than the non-remission cases at 72.9%

Table 2. Clinical management and pregnancy outcomes of the hospitalized patients.

	J	LH	H	NA	S	N	K
Antivirals	Oseltamivir, 2 x 75 mg	(-)	Oseltamivir, 1 x 75 mg	Oseltamivir, 2 x 75 mg	(-)	Oseltamivir, 2x 75 mg	Oseltamivir, 2x 75 mg
Antibiotics	Levofloxacin, 1 x 750 mg	Moxifloxacin, 1 x 400 mg	Meropenem, 3 x 1 g	(-)	Levofloxacin, 1 x 750 mg	Moxifloxacin, 1 x 400 mg	Meropenem, 3 x 1 g
Anticoagulants	Heparin, 350 units/hour	(-)	Heparin, 300 units/hour	(-)	(-)	Heparin, 500 units/hour	Heparin, 400 units/hour
Delivery	SC	not delivered	SC + IUD	not delivered	SC	Labor induction using misoprostol	SC
Delivery Complications	(-)	(-)	(-)	(-)	Uterine atony, SVH due to failed conservative surgery	(-)	(-)
Newborn	Girl, 1,960 g, 46 cm, AS: 7–8, survived	IUFD, not delivered	Boy, 1,615 g, 36.5 cm, AS: 1–1–1, death in hours	IUFD, not delivered	Boy, 2,655 g, 49 cm, AS 3–5, survived	IUFD: Girl, 1,000 g, 39 cm, AS 0	Girl, 2,335 g, 44 cm, AS 3–5–7, survived
Admission to ICU	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Ventilator	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Length of ICU stay (days)	3	4	8	5	5	7	4

($p<0.001$). On the other hand, prolonged oseltamivir therapy did not improve the patient's condition. In our hospital, oseltamivir was used at the beginning of the pandemic because of its availability and theoretical background. However, after many reports about the ineffectiveness of this drug and the entry of various new drugs, the use of oseltamivir for COVID-19 therapy was replaced by remdesivir (Beigel et al. 2020).

Antibiotics are not routinely given in COVID-19 cases. The medications are only administered in COVID-19 cases with superimposed infections, particularly bacterial pneumonia. The antibiotics used in our cases were meropenem, levofloxacin, and moxifloxacin. Currently, the antibiotics recommended for treating bacterial pneumonia in pregnant women are from the macrolide group and may be added with beta-lactam in severe disease conditions (Nasrallah et al. 2022). Both of the drugs have good safety profiles in pregnancy (FDA class B). Meropenem is a broad-spectrum beta-lactam antibiotic used in severe infections. In this study, there were two patients who received meropenem antibiotic therapy. Meanwhile, four other patients received therapy with antibiotics from the fluoroquinolone class. The fluoroquinolone class of antibiotics is highly effective against bacterial pneumonia. Fluoroquinolones have a bactericidal effect by inhibiting DNA synthesis and interacting with DNA gyrase and topoisomerase IV in cells. However, the use of fluoroquinolones raises concerns about their safety in pregnancy. Current clinical data indicate that the fluoroquinolone class is relatively safe to use in pregnancy and poses a low risk (Yefet et al. 2014). The FDA categorizes this class of drugs into category C, which means the benefits of administration outweigh the risks, and it

Table 3. The maternal laboratory results from all cases.

	J	LH	H	NA	S	N	K
Hb (mg/dL)	10.7*	11.4	12.7	10.4*	13.7	9.5*	11.3
Leukocyte (x 10^9/L)	10.52	15.91*	2.97*	6.9	26.6*	7.01	16.9*
Thrombocyte (x 10^9/L)	228	241	252	159	391	268	287
Hematocrit (%)	32.4	32.9	36.3	31.9	41.2	28	33.8
NLR	6.16	N/A	2.82	N/A	1.74	6.67	23.25
Random blood glucose (mg/dL)	109	120	216*	N/A	N/A	219*	N/A
BUN (mg/dL)	5.1	3.2	5.3	5.2	10	4.2	13.6
Serum creatinine (mg/dL)	0.18	0.27	0.67	0.41	0.53	0.49	1.18
Albumin (g/dL)	N/A	2.19*	3.16*	3.1*	3.26*	3.05*	2.8*
ALT (U/L)	N/A	64*	68*	33	21	44*	215*
AST (U/L)	N/A	42	34	19	10	44	215*
Natrium (mEq/L)	138	132*	135	137	140	135	130*
Kalium (mmol/L)	3.5*	3.1*	4.3	3.2*	4.6	3.6*	4.9
Chloride (mEq/L)	108	109	108	110	111	111	105
PPT (seconds)	N/A	10	11.1	12.6	11.4	11.8	N/A
APTT (seconds)	N/A	27	26.6	34.3	32.8	35.1	N/A
Procalcitonin (ng/mL)	N/A	0.15	0.41	0.3	2.78*	0.24	1.45
CRP (mg/L)	N/A	114.2*	68.86*	64.61*	N/A	78.47*	219.4*
Ferritin (mcg/L)	N/A	128.7	N/A	415	N/A	309.5	N/A
IL-6 (pg/mL)	N/A	N/A	44.67*	N/A	N/A	75.77*	N/A
D-dimer (µg/mL)	2.9*	9.75*	1.1*	2.59*	8.29*	1.28*	6.2*

BUN: Blood urea nitrogen; ALT: Alanine transaminase; AST: Aspartate aminotransferase; PTT: Partial thromboplastin time; APTT: Activated partial thromboplastin time.

Table 4. Daily monitoring of oxygen saturation in all cases (%).

	Admission	D+1	D+2	D+3	D+4	D+5	D+6	D+7	D+8	D+9	D+10
J	97	93	100	70							
LH	100	95	94	95	70						
H	95	98	89	100	99	93	98	98	97	97	70
NA	98	96	96	93	95	94	87	70			
S	55	93	90	86	92	85	81				
N	90	83	99	97	96	90	82	70	53		
K	91	92	90	85	100	98	92	83	78		

Notes: Green: Using simple oxygen mask. Blue: Using non-rebreathing oxygen face mask. Yellow: Using ventilator. Red: Maternal death.

may be used in pregnancy.

All patients in this study had elevated D-Dimer levels (>0.5 µg/mL), indicating a poor prognostic in patients with COVID-19. Unfortunately, not all patients received anticoagulant therapy (heparin). The reason was that all cases occurred at the beginning of the pandemic, when the guidelines for the management of patients with COVID-19, especially in pregnancy, were not yet established. As of now, we know that COVID-19 causes hypercoagulability, and the cytokine storm will cause abnormal clot formation and platelet hyperactivation (Miesbach & Makris 2020). Tang et al. (2020) reported that elevated D-dimer levels in COVID-19 patients were a predictor of mortality. In their study, mean D-dimer levels were found to be higher in the group who died than in the survivors of COVID-19 (2.12 vs. 0.61 µg/mL). Another study showed that elevated D-dimer levels (>1 µg/mL) increased the risk of death in COVID-19 patients (OR=18.4; 95% CI=2.6–128) (Zhou et al. 2020).

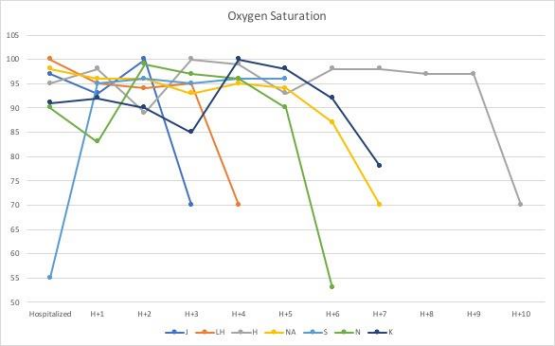

Figure 1. Progression of oxygen saturation during treatment.

Four patients delivered their babies by SC, and one patient delivered vaginally with misoprostol induction. Two patients were unable to deliver, i.e., Mrs. LH due to unviable gestational age (22 weeks) when maternal deterioration occurred and Mrs. NA due to the rapid worsening of the maternal condition. There is no evidence from scientific studies to suggest that one method of delivery is superior to another. In three out of four cases, the indication for SC was deteriorating COVID-19 symptoms, and ventilators were required during the procedure. The purpose of SC is to improve the condition of the mother by reducing the physiological burden of pregnancy. Obesity was found to be one of the risk factors for pre-eclampsia (Kartika et al. 2018). In the case of Mrs. S, SC was performed because of severe pre-eclampsia indications, gestational age, and the location of the breech. There were only three babies that survived from the total of seven cases (42.8%). This happened despite a previous study stating that all pregnancies with COVID-19 infections have a low perinatal mortality rate (2 of 108) (Pacheco et al. 2020, Zaigham & Andersson 2020). In cases of pregnancy with severe COVID-19 manifestations, the perinatal mortality rate appears to increase significantly. The cause of death in these conditions is chronic uteroplacental insufficiency or hypoxia due to prolonged maternal hypoxia, accompanied by prematurity. All infants who died were born at <32 weeks gestation.

The results from the laboratory tests during admission can be seen in Table 3. Almost all patients in these cases suffered from multiple organ dysfunctions, which were visible in the laboratory results. Only renal functions did not show any abnormalities in all cases. The CRP, IL-6, and D-Dimer levels significantly increased, although these markers were not always examined in all cases. Table 4 demonstrates the oxygen saturation progression day by day. Three methods were used as respiratory support, i.e., simple masks, non-rebreathing masks (NRM), and ventilators. On average, the patients experienced respiratory deterioration from days 2–4 before death. The exceptions were Mrs. L and Mrs. S, who suffered very fast respiratory failure on the day of the death. Oxygenation support is one of the most important factors in treating COVID-19 patients. Pregnant women require a higher minimum oxygen saturation level than non-pregnant women due to the increase in oxygen demand and oxygen partial pressure that occurs during pregnancy. In the care of pregnant women with COVID-19, oxygenation must be maintained with a minimum oxygen saturation of 96%. In this study's cases, the use of ventilators failed to reach the expected oxygen saturation minimum target. This indicated that severe lung damage and respiratory failure caused by COVID-19 might eventually become the leading cause of death in all cases (Dewantiningrum et al. 2023).

Strength and limitations

This case series illustrates the severe clinical progression of COVID-19 in pregnant women during the earliest phases of the pandemic, when all pregnant women had not yet been vaccinated against COVID-19. The strength of this case series is that it accurately reflects the natural clinical progression of COVID-19 during pregnancy in the absence of vaccination. These cases demonstrate that pregnancy is a major risk factor for severe and fatal COVID-19 infections. After the government initiated a vaccination program, maternal mortality and morbidity caused by COVID-19 decreased significantly. This highlights the significance of vaccination for expectant women. In these cases, we can also learn about the antibiotic, antiviral, and anticoagulant treatments administered during the

initial phase of the pandemic. Access to mechanical support in a timely manner is crucial for increasing the survival rate of expectant women suffering respiratory distress due to the COVID-19 infection. Although the use of retrospective data placed limitations on this study, the information can be obtained completely from the medical records.

CONCLUSION

This study provided evidence of the significant risks posed by COVID-19 during pregnancy, particularly for unvaccinated women. The rapid progression of the disease can lead to acute respiratory distress syndrome due to severe pneumonia, which requires mechanical ventilation. In addition, severe manifestations of COVID-19 can have adverse effects on perinatal outcomes, including stillbirth and fetal growth restriction. In order to mitigate the severe clinical manifestations of COVID-19, vaccination is crucial for pregnant women.

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Conflict of interest

None.

Funding disclosure

None.

Author contribution

MIAA was responsible for the design of the study, the acquisition, analysis, and interpretation of the data, the drafting and revision of the manuscript, the final approval of the manuscript, and all aspects of the work related to the accuracy and integrity of any part of the work. PM was responsible for the acquisition of the data, revision, and final approval of the manuscripts. MPW was responsible for the acquisition and analysis of the data, as well as the final approval of the manuscript. KEG and E were responsible for the acquisition, analysis, and interpretation of the data, as well as the revision and final approval of the manuscript. ER, MACL, and JYA were responsible for the acquisition of the data, revision, and final approval of the manuscript. HTJ, MAB, and BAT were responsible for the revision and final approval of the manuscript.

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Systematic Review

CALCIFEROL STATUS AND CLINICAL OUTCOMES IN CHILDREN WITH CONGENITAL HEART DISEASE AFTER HEART SURGERY

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ABSTRACT

Calciferol is a micronutrient and a secosteroid hormone that plays a role in maintaining the health of bones and soft tissues in the body, such as the myocardium, as well as the immune system. Hypovitaminosis D has been reported to be associated with poor clinical outcomes and child mortality in pediatric intensive care units. Children with congenital heart disease (CHD) are vulnerable to critical conditions and require early intervention due to heart failure. This study aimed to investigate the influence of calciferol status on the clinical outcomes of pediatric CHD patients who underwent heart surgery. A systematic literature review was conducted using the electronic databases from PubMed, Elsevier, and Cochrane. This study included observational and randomized control studies that assessed the calciferol status of pediatric CHD patients undergoing cardiac surgery. From a total of 168 studies, 8 studies were selected for review. The preoperative and postoperative calciferol status as well as clinical outcomes following pediatric cardiac surgery were reviewed. According to the findings, most pediatric CHD patients suffered from calciferol deficiency prior to corrective heart surgery, which further decreased postoperatively and was associated with clinical outcomes in the intensive care unit (ICU). Preoperative calciferol supplementation has been reported to enhance serum calciferol levels and is associated with good clinical outcomes in pediatric patients undergoing cardiac surgery.

Keywords: Calciferol; congenital heart disease; child cardiac surgery; child mortality

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Highlights:
1. Calciferol has cardioprotective properties, and a severe deficiency of calciferol is associated with septic shock, a longer duration of mechanical ventilation and treatment in the intensive care unit, and mortality.
2. The role of calciferol in critical conditions has been described in several research reports, but relatively little is known about its function in pediatric cardiac surgery.
3. The information presented in this systematic review is quite beneficial, as the sources cited are relatively recent and pediatric cardiac surgery will continue to develop in the future.

INTRODUCTION

Congenital Heart Disease (CHD) was estimated to occur in 17.9 of 1,000 children worldwide, with 19.1 of 1,000 boys and 16.6 of 1,000 girls. Ventricular septal defects (VSD) and atrial septal defects (ASD) accounted for about 29.6% of all cases. The prevalence of CHD in urban areas was 34.17% higher than in rural areas. Atrial septal defect (3.07 per 1,000 births), patent ductus arteriosus (PDA) (1.62 per 1,000 births), ventricular septal defect (1.18 per 1,000 births), tetralogy of Fallot (TOF) (0.62 per 1,000 births), and atrioventricular septal defect (0.47 per 1,000 births) are the five most

common subtypes (Morgan et al. 2013, Zhang et al. 2022). Epidemiological data in Indonesia are quite varied. Reports from Dr. Sardjito Central General Hospital, Sleman, Indonesia, revealed that the prevalence of CHD was 134 out of 10,000. Ventricular septal defects were the most common lesion among children (30%), followed by atrial septal defects (17%), patent ductus arteriosus (16%), and tetralogy of Fallot (7%). A study on the characteristics of infants born with CHD found that patent ductus arteriosus was present in 76.1% of the infants. Ventricular septal defects, atrial septal defects, and transposition of the great arteries were the most prevalent conditions following patent ductus arteriosus. About one in four infants with CHD are critically ill. Infants with critical CHD generally require surgery or other procedures in the first year of life. The lethality associated with critical CHD was 64.7%, with a mortality rate of 12.0%. The survival rates decreased by almost 70% in newborns with congenital heart disease, where the main cause of death was cardiogenic shock, respiratory tract infections (such as pneumonia and bronchiolitis), and malnutrition (Damayantie et al. 2019).

CHD management includes surgical procedures and transcatheter intervention, mostly performed within the first year of life. Most patients are in critical condition before and after the procedure. The defects and malformation abnormalities accompanied by comorbid diseases may worsen the child's condition. The condition of the child undergoing cardiopulmonary bypass (CPB) can lead to a critical condition. Some factors related to the critical condition post-cardiopulmonary bypass are young age, malnutrition, and congestive heart failure. The long duration of cardiopulmonary bypass and the complexity of the correction surgery are some of the intraoperative factors contributing to CHD mortality. In addition, the conditions of multiorgan dysfunction, bleeding, acute renal failure, and postoperative septic complications are also causes of death post-surgery (Morgan et al. 2013, Mamikonian et al. 2014, Guzzetta et al. 2015).

Vitamin D, also known as calciferol, is a micronutrient secosteroid hormone with two main forms, i.e., vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Generally, vitamin D is synthesized in the skin and obtained from animal-source foods. Calciferol, along with calcium, is important for bone health and the body's soft tissues, such as the myocardium and immune system. In the immune system, calciferol promotes the expression of an anti-inflammatory cytokine, i.e., interleukin 10 (IL-10). It also activates toll-like receptors that induce cathelicidin and β-defensin, which then start the autophagy activity of pathogens. Calciferol enhances the innate immune response that prevents cytokine storms by reducing the production of inflammatory cytokines in patients with viral infections and sepsis (Chun et al. 2014, Kumar et al. 2021, Ao et al. 2021). In the cardiac myocardium, calciferol was reported to be beneficial as a cardioprotector in dilated cardiomyopathy and heart failure. There was a significant correlation between low calciferol and heart failure. Another study also reports the role of these vitamins in improving B-natriuretic peptide levels and the quality of life in heart failure patients (Priya et al. 2016, Moretti et al. 2017).

Hypovitaminosis D, or vitamin D deficiency, is indicated by a concentration of <50 nmol/L (<20 ng/mL). Severe vitamin D deficiency with a 25-hydroxyvitamin D (25-OHD) concentration of <30 nmol/L (12 ng/mL) dramatically increases the risk of infection, mortality, and other diseases (Yeşiltepe Mutlu & Hatun 2018, Wei et al. 2018, Amrein et al. 2020). Previous studies also showed that calciferol deficiency status has been associated with critical conditions in children receiving intensive care. There was some evidence of severe vitamin D deficiency associated with septic shock, longer mechanical ventilation, and longer treatment in the intensive care unit (ICU). Conditions of severe respiratory tract infection, sepsis, and significant mortality risk were also associated with findings of 25-OHD deficiency status (<50 nmol/L) (Cariolou et al. 2019, Sankar et al. 2019).

Vitamin D supplementation in doses of 400 IU per day is recommended during infancy. The average needs of older children and adolescents (400–600 IU per day) are usually met from food and natural sources such as sunlight. However, in conditions of severe calciferol deficiency and rickets, oral cholecalciferol at a daily dose of 2,000 IU for children under 1 year old and 3,000 IU for older children can be given for 3 months. High doses of vitamin D supplements are not associated with an increased risk of severe side effects in children aged 0–6 years. Vitamin D supplementation for children in the dosage range of 1,200 to 10,000 IU/day and bolus doses up to 600,000 IU are also well tolerated (Gupta et al. 2022, Brustad et al. 2022). A single dose of vitamin D (150,000 IU) may increase 25-OHD levels and lower the incidence of septic shock in children with critical sepsis. Vitamin D supplementation also improves the concentrations of various sepsis-related cytokines, e.g., angiotensin II (Ang-II), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α). In a previous study, the cardiovascular sequential organ failure assessment (CV-SOFA) scores (1.76±0.8 vs. 2.3±1.1) and septic shock incidence (7% vs. 20%) were lower in the group with vitamin D supplementation compared to the control group, although the duration of mechanical ventilation and mortality were the same

between the two groups (Wang et al. 2020).

Clinically, pediatric patients with CHD will experience critical conditions due to anomalies that interfere with heart function. Some CHD patients experience heart failure and are in critical condition after undergoing cardiac surgery with cardiopulmonary bypass, affecting the outcome of the procedure. Earlier studies had reported on changes in vitamin D status before and after pediatric heart surgery, but the benefits of vitamin D supplementation in pediatric heart surgery were not widely explored (Sankar et al. 2019, Wang et al. 2020). Our study objectively aimed to report the changes in vitamin D status in children with CHD undergoing heart surgery and the benefits of vitamin D supplementation to assist in the clinical evaluation of shock events, additional fluid resuscitation volumes, inotropic vasopressor support, and other conditions that influence patients' outcomes.

MATERIALS AND METHODS

A search for literature was conducted on the medical databases, including PubMed, Elsevier, and Cohrane, with the keywords "calciferol", "vitamin D", "child heart surgery", and "child cardiac surgery outcome". The selected studies were journal articles published in English between January 2013 and December 2022. The inclusion criteria were open-access research publications, including observational studies, cohort studies, and clinical trials in humans with patients aged <18 years undergoing cardiac surgery with cardiopulmonary bypass due to CHD. The selected studies must discuss the interventions and comparisons of calciferol administration as well as the significance of serum vitamin D (calcitriol) levels before and after cardiac surgery (Page et al. 2021). Figure 1 shows the selection of the studies using the PRISMA flowchart.

Initially, the identification of the studies was performed by filtering the titles and abstracts before obtaining the full-text articles. Afterwards, a screening of the interventions, comparisons, characteristics, and output results of each study was carried out (Gupta et al. 2018). There were 326 pieces of research, but 8 duplicates and 87 studies not meeting the inclusion criteria were excluded. There were 68 studies in the follow-up screening, but 48 were considered irrelevant. For the remaining 20 pieces of research, 6 studies lacked detail, and 6 studies were systematic reviews as well (Siddaway et al. 2019). Finally, there were 8 eligible studies for this systematic review. The results and flow of the literature search are presented in Table 1.

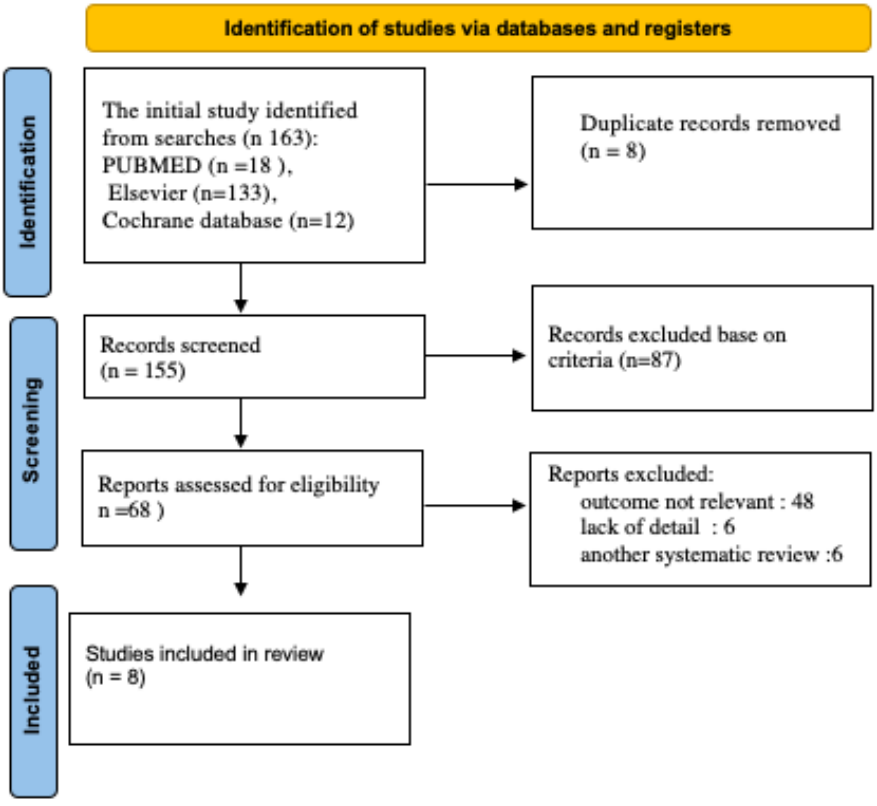

Figure 1. PRISMA flow diagram for the literature search.

Table 1. Characteristics and outcomes of the selected studies.

Authors, Year	Study Designs	Populations	Interventions/ Comparisons	Outcomes
McNally et al. (2013)	Prospective cohort	58 children aged <18 years with CHD who underwent elective and semi-elective cardiac surgery	Comparison of 25-OHD levels preoperatively, intraoperatively, and postoperatively; correlation of vitamin D status with clinical outcomes, including catecholamines, inotropic score, fluid boluses, total fluid intake, intubation duration, and PICU length of stay	CHD patients had low preoperative vitamin D levels and significant intraoperative decline. Low levels of 25-OHD were associated with higher fluid volumes and longer intubation.
Graham et al. (2013)	RCT	900 children with CHD who underwent cardiac surgery	Comparison of 25-OHD levels prior to surgery, during CPB, and 24 hours postoperatively; association of vitamin D status with clinical outcomes	Vitamin D deficiency was common in CHD patients, and lower postoperative vitamin D levels were associated with higher inotropic support in neonates after cardiac surgery.
Abou Zahr et al. (2017)	Prospective observational	20 CHD child patients aged between 2 months and 17 years who underwent CPB	Evaluation of serum 25OHD levels before, immediately after surgery, and 24 hours after surgery	CPB decreased the 25-OHD level by reducing the free fraction.
Acharya et al. (2018)	Cohort	56 children with CHD who underwent surgery and calcitriol evaluation preoperatively and postoperatively	Comparison of calcitriol levels preoperatively, postoperatively, and 48 hours after surgery	CHD patients had low levels of calcitriol intraoperatively and experienced a challenge in increasing the levels. Cholecalciferol reduced the severity of postoperative illness.
Ye et al. (2021)	Cohort	900 children with CHD who underwent cardiac surgery	Vasoactive inotropic score comparison of 25-OHD levels at 20–30 ng/mL and <20 ng/mL 24 hours after surgery	Vitamin D levels in child patients before cardiac surgery were associated with postoperative inotropic support 24 hours after the surgery.
Dohain et al. (2020)	Prospective cohort	69 children with CHD who underwent elective cardiac surgery with CPB	Comparison of vitamin D deficiency with levels of <20 ng/mL	Low levels of vitamin D after heart surgery were associated with inotropic support.
Sahu et al. (2019)	RCT	60 children with a TOF diagnosis and vitamin D levels of <20ng/dL	Children with CHD who underwent cardiac surgery and received vitamin D supplementation of 10,000 units/kgbw	TOF was highly associated with vitamin D deficiency after CPB. Vitamin D supplementation was useful to raise serum levels before and after surgery.
McNally et al. (2020)	RCT	41 children aged between 36 weeks and 17 years with CHD who underwent cardiac surgery, i.e., 21 received high doses of vitamin D vs. 20 received standard care	Comparison of preoperative cholecalciferol treatment with lower doses (400 IU/day for children aged <1 year; 600 IU/day for children aged >1 year) and higher doses (1,600 IU/day for children aged <1 year; 2,400 IU/day for children aged >1 year)	Daily vitamin D supplementation in high doses improved 15-OHD status before and after surgery in the PICU.

CPB: Cardiopulmonary bypass; CHD: Congenital heart disease; 25-OHD: 25-hydroxy-vitamin D; RCT: Randomized controlled trial; TOF: Tetralogy of Fallot; PICU: pediatric intensive care unit

RESULTS

Children with CHD and the calciferol status

There were 163 studies obtained from the literature search. After screening and excluding ineligible studies, eight studies were suitable to be reviewed in this study. There were five observational cohort studies and three randomized controlled trials (RCTs) that reported calciferol status in children with CHD who underwent open cardiac surgery (Figure 1).

Cardiac surgery performed on children aged <18 years with CHD significantly lowered calciferol levels over time until post-surgery (Table 1). A study observed the calciferol status of 58 children with CHD before surgery, during ICU admission, and over 4 hours to 2 days after surgery. There was a significant decrease in vitamin D levels after the heart surgery. There was a significant decrease in vitamin D levels after the heart surgery. In the study, the average preoperative calciferol measured with serum 25-OHD was 58±22.4 preoperatively. The average level changes from 34.2±14.5 nm when the patients entered the pediatric intensive care unit (PICU) to 34.2±14.5 four hours post-surgery, 33.9±13.6 one day later, and 36.5±12.2 after two days. The comparison of 25-OHD levels before and after surgery was significantly different (58.0 vs.

34.2 nm, p<0.001) (McNally et al. 2013).

Another study conducted on neonates with CHD also found that calciferol deficiency was present in 84% of the neonates who underwent cardiac surgery. Most neonates had low calciferol levels of <20ng/dL (Graham et al. 2013). A study by Abou Zahr et al. (2017) reported similar findings. Cardiopulmonary bypass decreased the 25-OHD levels of pediatric patients. The average levels of 25-OHD were measured before, during, and 24 hours after the cardiopulmonary bypass, with mean levels of 26.8±4.2 ng/mL, 21.5±5.7 ng/mL, and 23.0±4.9 ng/mL (p<0.001), respectively. In the group of patients with initial vitamin D deficiency, there was also a significant decrease, with an average of 21.3±8 ng/mL preoperatively, 19±5.8 ng/mL during surgery, and 19.5±6.6 ng/mL postoperatively.

Comparison of calciferol status and clinical outcomes in children with CHD who underwent cardiac surgery

Several studies on the calciferol status and clinical outcomes after pediatric heart surgery described the various hemodynamic parameters, the need for a ventilator, and the duration of treatment in the PICU (Table 1). McNally et al. (2013) reported that pediatric patients with lower calciferol levels of <25nm/dL required more catecholamine, postoperative fluid boluses, and fluid intake, had a prolonged intubation duration, and had a higher inotropic score compared to children with higher calciferol levels. After pediatric heart surgery, there were changes in calciferol levels according to a prospective observation by (Acharya et al. 2018). There was a significant decrease in intraoperative levels of 25-OHD (calcitriol) and a challenge to increase the levels postoperatively. The average calcitriol level was 122.3±69.1 pmol/L preoperatively and dropped to 65.3±36.5 pmol/L during PICU admission (p<0.0001). The majority (61%, n=34) of the patients were unable to increase their calcitriol levels within 48 hours after surgery. Postoperative predisposition from calcitriol deficiency was found to be inversely proportional to cardiovascular dysfunction, fluid requirements, ventilation support, and duration of PICU treatment (p<0.01).

A secondary analysis in the study by Acharya et al. (2018) showed the negative effects of CHD surgery on the calciferol axis. There was a strong association between a downward trend in calcitriol levels and the severity of the postoperative condition. In another study, calciferol levels were significantly related to the vasoactive inotropic score at a maximum of 24 hours after surgery and a longer cardiopulmonary bypass duration. Calciferol levels of <30 ng/mL were more likely to result in a vasoactive inotropic score of >15 compared to higher calciferol levels. Longer cardiopulmonary bypass duration was also found to be more prevalent among children with calciferol levels of <20 ng/mL. The association between calciferol status and clinical outcomes was still unclear. However, a study suggested that calciferol status was related to a patient's myocardial and inflammatory status (Ye et al. 2021).

In a study by Dohain et al. (2020), calciferol deficiency was common in pediatric patients with CHD. Most patients had calciferol deficiency after surgery, with an acute decrease in serum 25-OHD following a cardiopulmonary bypass. Lower levels of calciferol in children after heart surgery were associated with the need to improve inotropic support. Patients with calciferol deficiency postoperatively, with 25-OHD levels of <20 ng/mL, were more likely to need higher inotropic support. In the postoperative follow-ups, it was discovered that patients with calciferol deficiency were more susceptible to surgical wound infections, required extracorporeal membrane oxygenation (ECMO) support, and had a higher mortality rate before being discharged from the hospital (Dohain et al. 2020).

Consideration of calciferol doses in children with CHD who underwent cardiac surgery

Calciferol deficiency status before and after child heart surgery had an association with clinical outcomes in pediatric patients. In terms of maintaining the calciferol status, calciferol supplementation was given to achieve perioperative success. A randomized controlled trial conducted by Sahu et al. (2019) studied 60 children under 18 years of age with a tetralogy of Fallot diagnosis. Most patients had serum calciferol levels of <20 ng/dl, which decreased further after undergoing intracardiac surgical correction and cardio-pulmonary bypass. The calciferol levels in children with tetralogy of Fallot berries were below the baseline (<20 ng/dL) preoperatively up to one day postoperatively. The group that received calciferol therapy in a single megadose of 10,000–400,000 units/kg showed calciferol levels of >20 ng/dL both during surgery and six hours up to one day after surgery compared to the control group. No significant difference was found in the laboratory parameters (e.g., parathyroid hormone, serum calcium, and urinary calcium creatinine ratio), clinical outcomes of inotropic support duration, ventilation duration, or ICU treatment duration. However, calciferol therapy significantly improved the postoperative calciferol status of the pediatric patients' hearts (Sahu et al. 2019).

Another randomized controlled trial conducted by McNally et al. (2013) reported that high-dose

supplementation of calciferol improved vitamin D status before surgery and reduced treatment time in the ICU. In the study, D3-calciferol was administered regularly at doses of 400 IU/day for children aged <1 year and 600 IU/day for children aged >1 year. In addition, higher doses of 1,600 IU/day for children aged <1 year and 2,400 IU/day for children aged >1 year were administered for comparison. The higher doses resulted in higher intraoperative calciferol levels (81.5±36.5) compared to the lower doses in the control group (55.5±13.8). There were no side effects of hypercalcemia or postoperative hypercalciuria. The pediatric clinical outcomes also showed that patients who received high-dose calciferol therapy required less mechanical ventilation and dyalisis when they had acute renal failure. These results supported a high-dose regimen capable of reducing calciferol deficiency without additional side effects or toxicity, as well as improving clinical conditions. Vitamin D supplementation provided better clinical outcomes after pediatric heart surgery (McNally et al. 2020).

DISCUSSION

This systematic review showed that, generally, children with CHD had a calciferol deficiency. This condition might be related to the preceding malnutrition before surgery and the side effects of the disease. In this review, we also explained that there was a significant decrease in calciferol levels as an effect of cardiac surgery procedures and the hemodilution in cardiopulmonary bypass. Cardiac surgery poses risks to pediatric patients and can place them in a critical condition if monitoring in the ICU is inadequate (Morgan et al. 2013).

In a number of study reports, calciferol deficiency status in children and adults was associated with clinical outcomes, e.g., the need for longer mechanical ventilation, prolonged ICU treatment duration, infection, and respiratory failure. In this review, the postoperative calciferol status of pediatric patients with CHD was correlated with the need for inotropic support and mechanical ventilation. In critical conditions, calciferol supplementation can be administered to suppress various inflammatory responses that may occur during treatment (Cariolou et al. 2019, Kusumajaya et al. 2021).

The results of this review are still unable to confirm the role of calciferol in terms of improving the postoperative status of pediatric patients' hearts. The reasons were that many other factors affect a patient's external outcomes, such as heart failure that accompanies pediatric anomalies or abnormalities, the duration of surgery, the duration of cardiopulmonary bypass, bleeding, postoperative hemodynamic disorders, and the risk of sepsis (Darren et al. 2022). In the end, various efforts must be made to maintain a good clinical condition. Previous studies showed quite positive benefits of supplementation in improving calciferol status preoperatively and postoperatively. A number of studies agreed to the administration of vitamin D therapy if a child's 25-OHD level is <20 ng/dL, with a recommended dose of 400–2,000 IU/day to increase the 25-OHD level to 30–50 ng/dL. This therapy should be carried out while monitoring potential side effects, such as hypercalcemia and hypercalciuria, which usually result from taking very high doses of calciferol over an extended period of time (Bouillon 2017, Pludowski et al. 2018, de la Guía-Galipienso et al. 2021).

Vitamin D supplementation can maintain optimal serum calciferol status before and after surgical correction, particularly in critical conditions due to CHD. Supportive results from the reviewed studies revealed the benefits of high doses of calciferol on the clinical outcomes of CHD patients. The findings indicated no incidence of severe side effects after pediatric heart surgery (Sahu et al. 2019, McNally et al. 2020). We suggest that further research may study calciferol administration with large doses and various timings of administration in correlation with other parameters, including pro-inflammatory cytokines, immune response, hemodynamics, side effects, and the postoperative clinical status of the child's heart.

Strength and limitations

Several research reports have discussed the role of calciferol in critical conditions, but not much has been described about pediatric cardiac surgery. This systematic review provides additional information regarding the role of calciferol in pediatric heart surgery. Other research reported that several factors also affect the outcomes of pediatric heart surgery, including previous heart failure, nutritional status, co-infection, intraoperative problems (e.g., surgery duration, bleeding, and the complexity of congenital heart anomalies), inflammation, and several other factors associated with mortality in the ICU. However, the information presented in this systematic review is quite useful, as the sources cited are relatively recent and pediatric cardiac surgery will continue to develop in the future.

CONCLUSION

Most pediatric congenital heart disease (CHD) patients had low levels of calciferol below normal, which further decreased following cardiac surgery and cardiopulmonary bypass. Calciferol deficiency is associated with increased inotropic requirements

and prolonged intensive care unit (ICU) stays compared to sufficient levels of calciferol. Preoperative calciferol administration can enhance serum calciferol levels above normal and potentially reduce the use of mechanical ventilation following heart surgery.

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Conflict of interest

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Author contribution

ZE, A, FM, H, and RP contributed to the conception and design of the study. ZE, FM, H, TR, FZ, and HK drafted the manuscript. ZE, TR, FZ, and HK contributed to the critical revisions and final approval. ZE, A, FM, H, and RP collected, assembled, and analyzed the data. All authors also contributed to the revisions of the manuscript.

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