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Original Research Report

SODIUM HYALURONATE EYE DROPS FOR COLLEGE STUDENTS WITH COMPUTER VISION SYNDROME IN INDONESIA

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ABSTRACT

The use of computers and mobile devices is increasing. Computers and mobile devices help our daily work or study. However, prolonged use of them may cause computer vision syndrome (CVS). Nowadays, CVS becomes a health problem for everyone working with computers or mobile devices including college students because it causes dry eyes. It may disrupt reading, doing professional work, or using a computer which is important for college students to complete academic tasks. Sodium hyaluronate can be used to overcome the dry eye problem due to CVS. To assess the effectiveness of sodium hyaluronate eyedrops on students suffering from CVS, pre- and post-administration of sodium hyaluronate was measured for two weeks. Inclusion criteria for this study were college students aged 20-35 years, using a computer for >2 hours a day, not using nonsteroidal anti-inflammatory drugs (NSAIDs), not consuming drugs, or having a disease that affects tear production and bearing no pregnancy. Parameters assessed include tear break-up time using the Tear Break-Up Time (TBUT) test, tear production using the Schirmer I test, the number of clinically subjective symptoms, and Ocular Surface Disease Index (OSDI) scores. Data were analyzed using student paired t-tests or Wilcoxon Rank Test. There were statistically significant differences before and after the TBUT (4.4 vs 6.7 seconds; p<0.0001); the Schirmer I Test (4 vs 6 mm; p<0.05), and the number of clinically subjective symptoms (3 vs 0 clinically subjective symptoms; p<0.0001). The OSDI scores did not show statistical differences before and after administration of sodium hyaluronate (27 vs 21; p>0.05), but there was a positive impact from moderate to mild dry eye. Sodium hyaluronate eyedrops can be used as one of the CVS therapy strategies for students suffering from CVS.

Keywords: *Computer vision syndrome; sodium hyaluronate; Tear Break Up Time test, Schirmer I test; clinically subjective symptoms; OSDI score, healthy lifestyle*

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Highlights:

1. Computer vision syndrome (CVS) that causes dry eyes becomes a health problem for college students.
2. Sodium hyaluronate eyedrops can be used as one of the CVS therapy strategies for students suffering from CVS.

INTRODUCTION

The use of computers is increasingly common in society nowadays. Computers help our daily activities and increase our productivity in workplaces, schools, universities, or elsewhere. However, there are also the negative effects of computers, especially for prolonged use. Computer vision syndrome (CVS) or digital eye strain (DES) can occur because of computer use for more than 2 hours a day. Symptoms include ocular or non-ocular such as asthenopia, tired eyes, irritation, burning, red eyes, dry eyes, blurred eyes, double vision or headache, and neck pain (Blehm et al. 2005, Reddy et al. 2013, Bali et al. 2014, Alamri et al. 2022). Furthermore, although not yet widely investigated, the use of mobile devices such as mobile phones or tablets may cause CVS (Kim et al. 2017, Jaiswal et al. 2019, Altalhi et al. 2020).

Nearly 60 million people suffer from CVS and millions of new CVS cases appear each year (Sen & Richardson 2007, Alatawi et al. 2022, Adane et al. 2022). Worldwide CVS prevalence is estimated at 25%-93%. With the increasingly common use of computers and devices in our daily activities from year to year, there is a potential decrease in work productivity and the quality of life due to CVS. CVS can cause problems in some daily activities such as reading (OR 3.64, 95% CI 2.45-5.40, p<0.0001); doing professional work (OR 3.49, 95% CI 1.72-7.09, p<0.001); using a computer (OR 3.37, 95% CI 2.11-5.38, p<0.0001); watching television (OR 2.84, 95% CI 0.05-7.74, p<0.04); driving during the day (OR 2.80, 95% CI 1.58-4.96, p<0.0001); and driving at night (OR 2.20, 95% CI 1.48-3.28, p<0.0001). CVS also causes poor sleep quality and depression (Miljanović et al. 2007, Patil et al. 2019, Park et al. 2019).

Nowadays, college students rely heavily on computers and smartphones for their academic tasks. Thus, CVS is common among them. In Malaysia, the prevalence of CVS reached almost 90% of college students. Almost similar prevalence was found among students in India and Saudi Arabia. The use of computers for more than 2 hours a day is a risk factor for CVS (Reddy et al. 2013, Logaraj et al. 2014, Al Rashidi & Alhumaidan 2017). The main cause of CVS is dry eye disease (DED). Dry eyes must be treated immediately because if ignored, the degree of dry eyes will continue to be burdensome so that the patient's quality of life will worsened (Gunawan IPFA et al. 2022). The main treatment for DED is artificial tear eyedrops (Blehm et al. 2005, Şimşek et al. 2018, Labetoulle et al. 2022). Sodium hyaluronate plays an important role in tissue development and wound healing and is effective for lubrication. The drug was effective in relieving the symptoms of DED in several studies (Cheema et al. 2012, Maheshwary et al. 2013, Park et al. 2017, Ang et al. 2017). In a small clinical trial, the drug was effective in relieving the symptoms of DED compared to normal saline (Lemp 2008). Previously, CVS was investigated on 150 bank employees in Indonesia (Kusumawaty et al. 2015). Where, this current study investigated CVS in college students in Indonesia and the effectiveness of sodium hyaluronate to ameliorate the symptoms of CVS.

MATERIALS AND METHODS

Pre- and post-studies were carried out to investigate the effectiveness of sodium hyaluronate in college students suffering from CVS. Cross-sectional time-limited sampling was used to obtain samples during August-October 2017. The inclusion criteria for the sample were as follows: college students aged between 20-35 years; using a computer for more than 2 hours a day; currently not taking nonsteroidal anti-inflammatory drugs or drugs that affect tear production; not having an ailment that affects tear production, and bearing no pregnancy at the time of the study. Informed consent was given to each study participant. Those who were diagnosed with CVS were given sodium hyaluronate eyedrop for two weeks. Measurements were carried out to identify tear break time using the Tear Break Up Time (TBUT) test, tear production using the Schirmer I test, as well as the number of clinically subjective symptoms, and Ocular Surface Disease Index (OSDI) score before and after the use of sodium hyaluronate eyedrop. Pre- and post-data were analyzed using SPSS version 17. Normality data were analyzed using the Kolgomorov-Smirnov or Shapiro-Wilk Test. Normal data were then analyzed with the paired t-test, while abnormal data were analyzed using the Wilcoxon Rank Test. The significance value less than 0.05 was considered statistically significant.

RESULTS

This study has obtained the ethical approval of the Medical Research Ethics Committee of Faculty of Medicine, Universitas Airlangga with number 241/EC/KEPK/FKUA2017. There were 78 students attending this study. A total of 66 students were diagnosed with CVS (87%). Their demographic data can be seen in Table 1.

Table 1. Demographic data of students participating in this study

	Characteristics	Number (n)
Sex	Male	8
	Female	58
Age	<25 years old	62
	25-45 years old	4
	>45 years old	0

Most of the participants were female and aged under 25 years old. Participants of young age for this study were selected as they more likely had an evaporative dry eye. Meanwhile, old people were not selected as they had reduced tear production because of hormonal causes.

The measured parameters of the effectiveness of sodium hyaluronate are shown in Figure 1, Figure 2, Figure 3, and Figure 4. Those figures show the analysis of the TBUT test, the Schirmer I test, and the number of clinically subjective symptoms. Those parameters were statistically significant ($p<0.05$). Only the OSDI score was not statistically significant ($p>0.05$). However, there was a trend of declining OSDI scores even though it was not significant statistically.

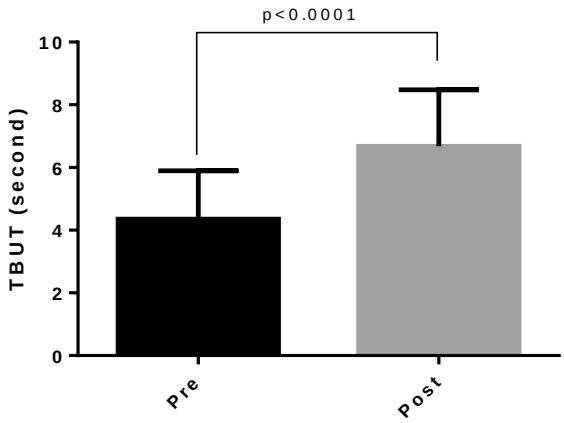

Figure 1. Pre- vs post-TBUT results

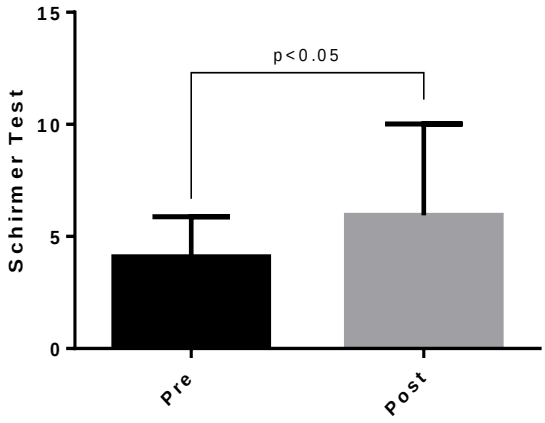

Figure 2. Pre- vs post-Schirmer I test results

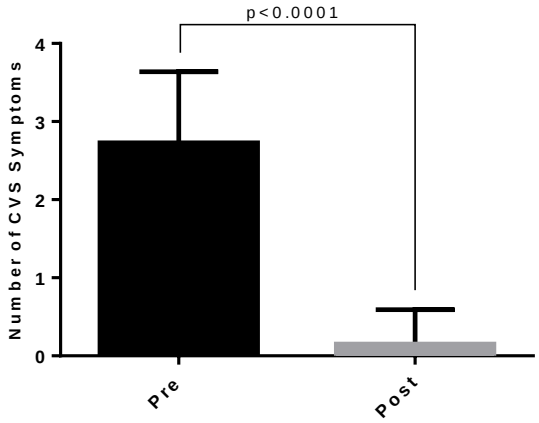

Figure 3. Pre- vs post-number of clinically subjective symptoms

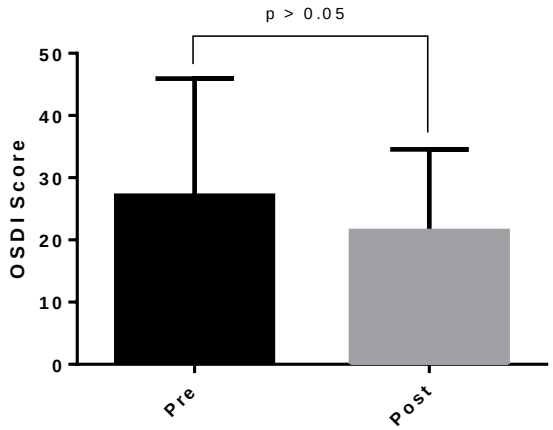

Figure 4. Pre- vs post-OSDI scores

DISCUSSION

The percentage of students suffering from CVS was 87%. This number was almost similar to the prevalence of CVS in college students in Malaysia, India, and Saudi Arabia (Reddy et al. 2013, Logaraj et al. 2014, Al Rashidi & Alhumaidan 2017, Altalhi et al. 2020, Iqbal et al. 2021).

The worldwide prevalence of CVS was estimated at 25%-93%. While there is trending use of computers and devices in our daily activities for something good, there is also a potential problem such as CVS for college students. Such problem may hinder them to complete academic tasks. The main cause of CVS is DED, and the effectiveness of the therapeutic strategy for CVS can be measured using some dry eye parameters. The main treatment of DED is artificial tear eyedrop (Blehm et al. 2005, Şimşek et al. 2018, Labetoulle et al. 2022)

The Tear Break Up Time (TBUT) is a parameter that measures the stability of tear film (Mcmonnies 2018, Hwang et al. 2020). It measures the time of tearing. DED patients have lower TBUT. A normal person has TBUT of >10 seconds. The TBUT before and after sodium hyaluronate administration showed an increase. The initial average before administration of sodium hyaluronate was 4.4 seconds. After two weeks of therapy, the TBUT value increased to an average of 6.7 seconds lower than the normal TBUT, 10 seconds. Longer administration of sodium hyaluronate may be able to return the TBUT value to normal. Another study also proves that sodium hyaluronate is better and faster than carboxymethylcellulose (CMC) (Groß et al. 2018). It is also safe for moderate and severe dry-eye patients (Maheshwary et al. 2013, Park et al. 2017). The efficacy of sodium hyaluronate eye drops was shown also in multi center trial (Cheema et al. 2012).

The Schirmer I test is a test to measure the tear production. The test was carried out by inserting a piece of Whatman filter paper into both eyes for five minutes. The wet paper length was measured in mm. It is considered normal when the result of Schirmer I test >10 mm. A CVS sufferer would show a test result of <10 mm or less for severe dry-eye person. The results of sodium hyaluronate administration are seen in Figure 2. The administration of sodium hyaluronate increased tear production according to the pre-Schirmer I test with average wet paper length of 4 mm and post Schirmer I test with average wet paper length of 6 mm ($p<0.05$). The Dry Eye Workshop (DEWS) dry eye severity grading scheme stated that the Schirmer I test result of <5 mm/5 minutes is categorized as dry eye level 3, whereas Schirmer I test result of <10 mm/5 minutes is categorized as dry eye level 2. This scheme categorizes dry eye levels from 1 (low) to 4 (severe) (McAlinden et al. 2017).

The next parameter for evaluating the effectiveness of sodium hyaluronate before and after administration is the number of clinically subjective symptoms expressed by the patient during the examination. In this study, the participants mentioned intra-ocular symptoms (blurred vision, painful eyes, eye strain,

watery eye, itchy eyes, double vision, tired eyes, and greasy eyes) or nonocular symptoms (headache, shoulders pain, and neck pain). Figure 3 shows a decrease in the number of clinically subjective symptoms of CVS patients after two-week therapy with sodium hyaluronate ($p<0.05$). The initial common clinically subjective symptoms include ocular or nonocular symptoms. After sodium hyaluronate administration, the clinically subjective symptoms reduced to almost zero symptoms.

Furthermore, the OSDI questionnaire is a common diagnostic tool for dry eye. It consists of 12 questions whose total OSDI score can be categorized as severe, moderate, mild, or normal. The score ranges from 0 to 100. The OSDI score of 0-12 is normal, 13-22 is mild, 23-32 is moderate, and >32 is severe. Figure 4 shows the results of administration of sodium hyaluronate in CVS patients by OSDI scores. In this study, the statistical results of the OSDI scores before and after therapy showed no significant difference ($p>0.05$), but the OSDI level of dry eye changed from moderate to mild (OSDI score from 27 to 21). Finally, even though artificial tear, such as sodium hyaluronate, is effective for DED due to CVS, other measures among mobile device users are widely advised, such as the 20-20-20 rule and ergonomic practices (Mowatt et al. 2018). These non-drugs measures are also important in preventing the occurrence of DED due to CVS.

Strength and limitation

This research can be useful for academics because it provides administration of Sodium hyaluronate to CVS by providing CVS therapy strategies. Further studies can be conducted based on the findings of this study to investigate the effect of Sodium hyaluronate among college students who have CVS on a larger scale and to prevent its prevalence .

CONCLUSION

Sodium hyaluronate administration in students suffering from CVS can be used as one of the CVS therapy strategies. The effectiveness of this therapy was measured from four parameters (i.e., TBUT, Schirmer I Test, clinically subjective symptoms, and OSDI Score).

Acknowledgment

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Conflict of interest

There was no conflict of interest.

Funding disclosure

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Author contribution

BSZ collected and analyzed the data, and wrote the manuscript. RL collected the data and wrote the manuscript. TA also collected the data and wrote the manuscript.

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Original Research Report

MORTALITY ASSESSMENT OF PEDIATRIC SEPTIC PATIENTS THROUGH PEDIATRIC SOFA+ANION GAP AND PELOD-2 SCORES

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ABSTRACT

Sepsis and septic shock are some of the causes of morbidity and mortality (50-60%) in pediatric patients treated in intensive care rooms. This study aimed to compare the accuracy of pediatric Sequential Organ Failure Assessment (pSOFA) score combined with anion gap (AG) score to Pediatric Logistic Organ Dysfunction-2 (PELOD-2) score in the assessment of mortality in pediatric septic patients at the Resuscitation Room of Dr. Soetomo Geeneral Academic Hospital, Surabaya, Indonesia. This was a retrospective observational cohort study using pediatric sepsis diagnosis guidelines based on the 2016 Pediatric Sepsis Consensus and medical records between January-December 2018. All data of patients aged 1 month to 16 years with suspected infection at the Resuscitation Room were collected based on predisposing infections, signs of infection, and warning signs. Organ dysfunction was assessed by calculating the pSOFA+AG scores, PELOD-2 scores, and corrected anion gap (cAG) in the first 24 hours. Sepsis mortality was assessed by comparing the results of the pSOFA, pSOFA+AG, and PELOD-2. The results showed 94.9% sensitivity and 70.0% specificity (p<0.0001) in the pSOFA, 89.9% sensitivity and 71.3% specificity (p<0.0001) in the PELOD-2, 79.7% sensitivity and 65% specificity (p<0.0001) in the AG, 79.7% sensitivity and 73.8% specificity (p<0.0001) in the cAG, and 79.3% sensitivity (p<0.0001) in the pSOFA+AG. In conclusion, pSOFA was more sensitive than PELOD-2, while the use of pSOFA+AG was not more sensitive than PELOD-2 in assessing the mortality of pediatric septic patients.

Keywords: *Sepsis; pediatric Sequential Organ Failure Assessment (pSOFA); anion gap (AG); Pediatric Logistic Organ Dysfunction-2 (PELOD-2); pediatric patient mortality; child mortality*

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Highlights:

1. Sepsis and septic shock cause morbidity and mortality in pediatric patients.
2. The accuracy of pediatric sequential organ failure assessment and anion gap (pSOFA+AG) was compared with AG and pediatric logistic organ dysfunction-2 (AG+PELOD-2).
3. The mortality assessment of pediatric septic patients showed that pSOFA was more sensitive than PELOD-2, while pSOFA+AG was not more sensitive than PELOD-2.

INTRODUCTION

Sepsis is a common phenomenon surrounded by the uncertainty of major public health problems in children throughout the world (Hunt 2019, Wong 2022). The Surviving Sepsis Campaign (SSC), which aims to improve clinical outcomes for patients undergoing sepsis treatment, developed and approved international clinical practice guidelines for the management of sepsis (Rhodes et al. 2017, Levy et al. 2018). These guidelines consist of bundles that combine treatments for different components of sepsis. Adherence to the SSC package has remained a cornerstone in improving quality and clinical outcomes for patients with sepsis since the publication of the first SSC guidelines (Levy et al. 2018, You et al. 2022). Although understanding pathophysiology and therapy has increased, sepsis remains the leading cause of non-cardiac mortality in Intensive Care Units (ICU) (Handayani, N., et al. 2022). The high mortality rate in sepsis is frequently due to delays in identification and treatment (Pasaribu, F.M., Setyaningtyas, A., & Andarsini, M. R. (2021).

Sepsis diagnosis using the 2001 definition by the Surviving Sepsis Campaign (SSC) was too sensitive (96.9% sensitivity) and less specific (58.3% specificity), resulting in high antibiotic resistance due to the high use of antibiotics, as well as increased expenditure on facilities and infrastructure (Kawasaki 2017, Costa et al. 2018, Rijal & Ramdhoni 2018).

In 2001, the Society of Critical Care Medicine (SCCM) defined sepsis in children as an infection with two or three signs of Systemic Inflammatory Response Syndrome (SIRS). In 2005, the definition of sepsis in children still maintained

the signs and symptoms of SIRS, in addition to organ failure. The validity of SIRS for identification and risk stratification of septic patients is questionable because tachycardia and tachypnea are adaptive mechanisms that often appear in pediatric patients with fever and infection, so they are not further investigated in research. In 2012, SIRS was defined as a condition resulted from infection found through positive culture; or a large suspicion of infection seen from physical, laboratory, and radiological findings. Sepsis is SIRS with evidence of infection, while severe sepsis is the presence of sepsis with organ dysfunction (Handayani & Nugrohowati 2022). Septic shock happens when septic patients' blood pressure decreases after adequate fluid resuscitation or if hemodynamics requires vasopressor support (Randolph & McCulloh 2014, Schlapbach et al. 2017).

The latest sepsis definition in 2016 emphasizes that sepsis is distinguished from uncomplicated infection by the presence of life-threatening organ failure resulting from the regulation system failure of the host response against infection. The Sequential Organ Failure Assessment (SOFA) score is used to assess organ dysfunction in the 2016 SSC guidelines. The SOFA score has variables that are easily measured, available, and routinely checked in the the intensive care unit (ICU). This latest definition of sepsis is expected to be widespread (Gogia & Prasad 2016, Schlapbach et al. 2017, Matics & Sanchez-Pinto 2017).

In February 2018, an Intensive Care Medicine study by Schlapbach et al. (2018) issued a multicenter binational cohort prospective study of organ dysfunction scores through SOFA, quick SOFA (qSOFA), and Pediatric Logistic Organ Dysfunction-2 (PELOD-2) among pediatric patients with infectious diseases admitted to the ICU. These scores were compared with the criteria of SIRS to distinguish hospital mortality or length of stay in the ICU. The SOFA and PELOD-2 scores were significantly more accurate than SIRS and qSOFA in predicting mortality. Seymour et al. (in Costa et al. 2018) found the same results from the use of SOFA score in the diagnosis of sepsis among adult patients.

The prevalence and mortality of pediatric sepsis have become comparable to figures reported from adult ICUs in high-income countries (Hartman et al. 2013; Schlapbach et al. 2015; Weiss et al. 2015). Defining sepsis in the absence of a gold standard remains a challenge (Angus 2016). According to the 2001 consensus statement of the Society of Critical Care Medicine, pediatric sepsis is defined as an infection in which at least two of the four criteria of SIRS are met (Carcillo & Fields 2002, Goldstein et al. 2005). The 2005 consensus definition of pediatric sepsis retained the SIRS requirement and provided more specificity for definitions of organ failure (Goldstein et al. 2005). The validity of the SIRS criteria in identifying and risk-stratifying adult patients with sepsis has been questioned because of its demonstrated insufficient sensitivity and specificity (Kaukonen et al. 2015; Raith risk-stratifying adult patients with sepsis has been questioned because of its demonstrated insufficient sensitivity and specificity (Kaukonen et al. 2015; Raith et al. 2017). On the other hand, tachycardia and tachypnea indicate adaptive mechanisms that accompany febrile infections in pediatric patients, which include patients suffering from diseases with near-zero mortality, e.g. bronchiolitis (Schlapbach et al. 2017). Therefore, the face validity, construct validity, and sensitivity of the SIRS criteria were not examined in many critically ill pediatric patients (Schlapbach et al. 2018). However, current definitions of pediatric sepsis remain essentially based on sepsis-2, which poses a major obstacle to research, bench-marking, coding, and quality control (Schlapbach 2017, Schlapbach & Kissoon 2018). The implementation of the clinical criteria for identifying individuals with sepsis is consistent with the definition of sepsis-3 , which is based on the SOFA score. However, neither SOFA nor qSOFA has been developed for children (Schlapbach et al. 2018).

In addition to the scoring system above, other parameters can be used to assess mortality, e.g. the anion gap (AG). A prospective observational cohort study by Pongmanee & Vattanavanit (2017) assessed biomarkers (base excess and AG) used in the emergency room septic shock patients, in which lactate and AG showed a strong relationship indicating that the biomarkers can be used in the initial assessment of septic shock patients, especially if there is a high cut-off point of 15.8 and 18.5 for AG. Another retrospective observational study by Sneha et al. (2022) using the consensus conference criteria found no correlation in the changing trends of anion and lactate among 130 severe septic shock patients (15-65 years) in the ICU. AG cannot be considered a substitute for lactate testing. Assessment of AG value to predict the mortality of patients in pediatric intensive care unit (PICU) showed that the corrected anion gap (cAG) can be used by combining with other scoring systems to produce better results (Pongmanee & Vattanavanit 2017, Kim et al. 2017).

A study on emergency department patients with sepsis by Adams (2006) found that AG and serum lactate are correlated, but not codependent. Berkman et al. (2009) studied 1,419 patients with septic shock from the Emergency Department of Boston Hospital and concluded that AG is a good but not excellent screening test to help identifying elevated lactate in emergency department population at risk of sepsis. Park et al. (2008) conducted a study at the Medical and Surgical ICU of the Hospital of the University of Sao Paulo, Brazil, in September 2004–November 2005 and

concluded that acidosis resolution is attributable to the decrease of strong ion gap and lactate level. Several studies have been conducted to test the validity of the use of SOFA scores in pediatric patients by modifying some age-adjusted variables known as pediatric SOFA (pSOFA) scores which can significantly predict the mortality output and prognosis of septic pediatric patients. This study aimed to compare the accuracy of the pSOFA score combined with AG score to the PELOD-2 score in the assessment of mortality in septic pediatric patients at the Resuscitation Room of Dr. Soetomo General Academic Hospital,Surabaya, Indonesia.

MATERIALS AND METHODS

This study was a retrospective observational cohort conducted at the Resuscitation Room of Dr. Soetomo Hospital from January to December 2018. The inclusion criteria of this study were critically ill patients with suspected infections, aged 1 month to 16 years, who were treated in the Resuscitation Room. The exclusion criteria of this study were patients aged less than 1 month and more than 16 years; patients with a history of trauma, kidney disease, and died less than 24 hours; and patients referred to other hospitals who had been treated and previously given care. The assessment of suspected infection was based on the predisposing factors to infection (age, nutritional assessment of suspected infection was based on the predisposing factors to infection (age, nutritional factors, comorbidities, and history of therapy), signs of infection (hyperthermia/hypothermia, tachycardia, focus of infection, leukocytes, platelets, CRP, procalcitonin), and warning signs (loss of consciousness, cardiovascular disorders respiration disorder). The organ dysfunction of each patient was assessed using the pediatric SOFA, AG, cAG, and PELOD-2 in the first 24 hours. Then, each predictor of the mortality of the pediatric septic patients were measured and compared.

RESULTS

There were 139 patients fulfilling the inclusion and exclusion criteria, consisting of 80 (57.6%) boys and 59 (42.4%) girls, with mortality rates of 33 (55.9%) in male and 26 (41.1%) in female. The average age of the patients who died was 36.3 months (3 years) with a normal distribution and a value of p<0.92. In this study, the most commonly found diagnoses were pneumonia, found in 79 (56.8%) patients with a mortality rate of 35 (59.3%); meningoencephalitis, in 23 (16.5%) patients with a mortality rate of 6 (10.2%); and encephalitis, in 15 (10.8%) patients with a mortality of 9 (15.3%) patients.

Table 1. Patient's characteristics

	Outcome			
	Alive (n=80)	Died (n=59)	*p*-value	OR (CI 95%)
Age (months)	11 (1 - 180)	12 (1-192)	0.92	1 (0.993-1.006)
1-11	42 (53.2%)	27 (45.0%)		
12-23	11 (13.9%)	11 (18.3%)		
24-59	10 (12.7%)	6 (10.0%)		
60-143	12 (13.9%)	11 (20.0%)		
≥144	5 (6.3%)	4 (6.7%)		
Gender			0.74	1.122 (0.595-2.215)
Male	47 (58.8%)	33 (55.9%)		
Female	33 (41.2%)	26 (44.1%)		
Suspected sepsis				
Infection predisposition	2.82±1.088	3.05±1.007	0.21	1.229 (0.888-1.703)
Signs of infection	4.075±0.882	4.115±0.931	0.54	1.125 (0.772-1.639)
Warning sign	2.437±0.672	2.813±0.392	0.001	3.593 (1.744-7.400)
Diagnosis			*	*
Pneumonia	44 (55.0%)	35 (59.3%)		
Meningoencephalitis	17 (21.2%)	6 (10.2%)		
Encephalitis	6 (7.5%)	9 (15.3%)		
GEA	3 (3.8%)	3 (5.1%)		
Peritonitis	4 (5.0%)	1 (1.7%)		
Mean Value				
Lactate	1.473±0.905	4.516±1.742	<0.0001	4.903 (3.016-7.969)
Anion gap (AG)	16.69±5.241	25.398±6.897	<0.0001	1.275 (1.175-1.385)
Corrected AG (cAG)	17.667±5.342	26.591±6.694	<0.0001	1.289 (1.184-1.404)
pSOFA	6.137±2.822	11.508±2.254	<0.0001	2.079 (1.651-2.617)
PELOD-2	4.162±3.074	9.576±4.022	<0.0001	1.617 (1.371-1.907)

* Distribution frequency value without distribution test. p<0.05 showed that data distribution was not normal.

Table 2. Predictor analysis

Predictor	Alive	Dead	*p* Value	OR	CI Interval 95%
PELOD-2					
<6	57 (71.2%)	6 (10.2%)	<0.0001	1.619	0.289-9.067
≥6	23 (28.8%)	53 (89.9%)			
pSOFA					
<8	56 (70.0%)	3 (5.1%)	<0.0001	7.854	1.650-37.384
≥8	24 (30.0%)	56 (94.9%)			
AG					
<18.5	52 (65.0%)	12 (20.3%)	<0.0001	0	0
≥18.5	28 (35.0%)	47 (79.7%)			
cAG					
<21	59 (73.8%)	12 (20.3%)	<0.0001	2.846	0.774-10.465
≥21	21 (26.2%)	47 (79.7%)			
Lactate					
<2.6	73 (91.2%)	5 (8.5%)	<0.0001	47.148	13.221-168.129
≥2.6	7 (8.8%)	54 (92.5%)			
pSOFA + AG					
<8+<18.5	95.2%	4.8%	<0.0001	76.6	16.1-363.3
≥8+≥18.5	20.7%	79.3%			

p<0.005: significant, logistic regression; Chi square

In this study, relations of PELOD-2, pSOFA, AG, cAG, lactate, and pSOFA+AG scores with mortality were analyzed using logistic regression and Chi-square tests. The cut-off points of PELOD-2 score was 6, pSOFA 8, AG 18.5, cAG 21, and lactate 2.6. Furthermore, the sensitivity and specificity values of each predictor were calculated and summarized in Table 3.

Table 3. Sensitivity and specificity values

Predictor	AUC	*p*-value	Sensitivity	Specificity	NPV	PPV
PELOD-2	0.881	<0.0001	89.8%	71.3%	90.5%	69.7%
pSOFA	0.924	<0.0001	94.9%	70.0%	94.9%	70.0%
AG	0.843	<0.0001	79.7%	65.0%	81.3%	62.7%
cAG	0.852	<0.0001	79.7%	73.8%	83.1%	69.1%
Lactate	0.934	<0.0001	91.5%	91.3%	93.6%	88.5%

p <0.005: significant, logistic regression; Chi Square

The receiver operating characteristic (ROC) chart shows that the largest predictor area is the lactate area and then followed by the p-SOFA score, PELOD-2 score, and cAG, while the smallest area is the AG area with p<0.0001.

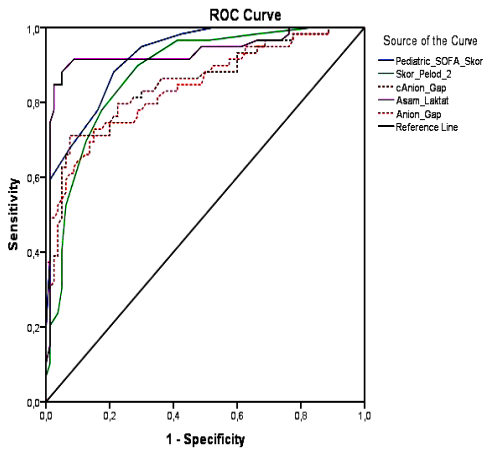

Figure 1. ROC chart of each predictor

In addition to analyzing the relationship between the predictors of mortality as described above, this study also analyzed the use of each predictor simultaneously (multivariate), so that its performance could be seen. Table 4 shows the results of the analysis.

Table 4. Performance of each mortality predictor

Predictor	Univariate			Multivariate		
	p-value	OR	CI 95%	*p*-value	OR	CI 95%
PELOD-2	<0.0001	1.6	1.3-1.9	0.58	1.6	0.3-9.1
pSOFA	<0.0001	2.0	1.6-2.6	0.01	7.8	1.6-7.4
AG	<0.0001	1.2	1.1-1.3	0.99	0	0
cAG	<0.0001	1.2	1.1-1.4	0.11	2.8	0.8-10.5
Lactate	<0.0001	4.9	3.0-7.9	<0.0001	47.1	13.2-168.1

p <0.005: significant, logistic regression; chi square

DISCUSSION

In this study, the highest distribution of age groups with sepsis diagnosis was children aged 1-11 months (49.6%). As stated by Kawasaki (2017), the incidence of sepsis in children is high, especially in infants (5.16 per 1,000), but decreases dramatically with age, especially at the age of 10-14 years. This is caused by the vulnerability of infants to infection, sepsis, and even to septic shock. In Indonesia, the incidence of sepsis is higher in the neonatal and infant group of less than 1 year compared to ages 1-18 years (9.7 versus 0.23 cases per 1,000) (Priyatiningsih et al. 2016).

In the findings of this study, the highest incidences of infection were caused by respiratory system infections (56.8%), central nervous system infections (27.3%), gastrointestinal infections (7.9%), and other infections (7.9%). Kawasaki (2017) reported similar findings that the highest sources of infection were from the bloodstream (67.8%) and respiratory tract (57.2%). In Indonesia, most patients with severe sepsis suffer from infections of respiratory tract (36-42%), bacteremia, and urinary tract (Priyatiningsih et al. 2016). A global study listed three most commonly found comorbid conditions that accompany pediatric patients with sepsis, i.e. the respiratory (30.3%), gastrointestinal (24.9%), and cardiovascular (24.0%) conditions (Weiss et al. 2015).

This study aimed to examine whether the use of pSOFA+AG compared with PELOD-2 was better in assessing the mortality of pediatric septic patients. The relationship between the predictors and mortality was the first analyzed in this study, which used the logistic regression statistical test and Chi-square test.

In the analysis of data obtained, if the PELOD-2 value was ≥6, the mortality rate was 89.8%. If PELOD-2 value was <6, then the mortality rate dropped to 10.2%,

with 0.881 area under the ROC curve (AUC), 89.8% sensitivity, 71.3% specificity, 0.697 positive predictive values (PPV), 1.6 odds ratio (OR), and <0.0001 p-value. From the results of this study, it was found that the PELOD-2 score had a significant relationship in assessing mortality.

The analysis of pSOFA scores showed a significant relationship to mortality with a p-value <0.0001, while the cut-off point of the pSOFA score from the logistic regression analysis was 8. The pSOFA score of ≥8 had a mortality rate of 94.9%. On the other hand, the pSOFA score of <8 decreased the mortality rate to 5.1%, with 0.924 AUC, 94.9% sensitivity, 70.0% specificity, 70.0% PPV, 7.8 OR, and <0.0001 p-value. This analysis showed that the pSOFA score had a strong and significant relationship with the mortality rate of pediatric septic patients.

This study also aimed to analyze the combined use of pSOFA and AG, so the relationship between the AG value and mortality was also studied. The results showed that the AG value in this study was significant for mortality with a p- value of <0.0001. The logistic regression test and Chi-square test resulted in the cut-off point value of the AG, which was 18.5. The AG value of ≥18.5 had 79.7% mortality rate, while the AG value of <18.5 had 20.3% mortality rate. When compared with pSOFA and PELOD-2, the mortality reduction from AG was not as large as those from pSOFA and PELOD-2. The analysis of AG values on mortality had an area of 0.84 AUC, 79.7% sensitivity, 65% specificity, 62.7% PPV, and <0.0001 p-value. From these results, the AG value showed lower sensitivity, specificity, and AUC when compared with pSOFA and PELOD-2.

The anion gap (AG) value is also influenced by albumin value. Therefore, the existence of hypoalbumin condition also affects the AG value. Most pediatric septic patients in this study had low albumin status, so the value of the cAG was also analyzed to recognize the mortality rate. The cAG value and mortality showed a significant relationship, with p-value <0.0001. The cut-off point value of the cAG was 21. The cAG value of ≥21 had 79.7% mortality rate. On the other hand, the cAG value of <21 decreased the mortality to 20.3%, with 0.852 AUC, 79.7% sensitivity, 73.8% specificity, 69.1% PPV, 2.8 OR, and <0.0001 p-value. This analysis showed that cAG was better in specificity, AUC, and PPV values compared to AG.

Lactate is a very significant and strong predictor of mortality. This predictor has been widely investigated and the results are all very significant. Therefore, this study also analyzed the relationship of lactate and mortality. This study showed a similar result as other studies that lactate had a significant relationshipwith mortality, with <0.0001 p-value and 4.9 OR. The lactate cut-off point in this study was 2.6. The lactate value of ≥2.6 had 91.5% mortality rate. On the other hand, the lactate value of <2.6 dropped mortality rate to 8.5%, with 0.934 RUC, 91.5% sensitivity, 91.3% specificity, 88.5% PPV, up to 47.1 OR, and <0.0001 p- value. Compared to the other predictors above, lactate was the best predictor in assessing mortality.

This study combined the use of pSOFA+AG and made the operational definition of this combination. The combination group was divided into for groups according to the cut-off point value, i.e. ≥8 pSOFA and ≥18.5 AG, <8 pSOFA and ≥18.5 AG, ≥8 pSOFA and <18.5 AG, and <8 pSOFA and <18.5 AG. The relationship between each group and its performance related to mortality was analyzed. The results of ≥8 pSOFA group and ≥18.5 AG were 79.3% mortality rate, with 76.6 OR, 95% CI of 16.1-363.3. As the p-values were <0.0001, the results were significant. The assessment of the performance of the pSOFA score itself resulted in 94.9% mortality rate with p<0.0001, indicating that the pSOFA was more sensitive compared to pSOFA+AG. The addition of AG did not improve the performance of pSOFA that was already good.

When analyzed separately (univariate), the performance of each predictor to mortality showed a strong and significant relationship, as seen in Table 4. On the other hand, if the predictors were analyzed simultaneously (multivariate) by seeing the dominance of each predictor, the pSOFA and lactate score were significant, in which pSOFA had a p-value=0.001 (OR 7.8, 95% CI 1.6-37.4), while lactate had a p-value<0.0001 (OR 47.1, 95% CI 13.2-168.1). In this study, the performance of pSOFA and lactate showed the two predictors as the best and most significant predictors compared to other predictors. The pSOFA scores were more sensitive than the PELOD-2 scores, meanwhile the addition of AG did not increase or improve the performance of the pSOFA score.

Strength and limitation

This study investigates the usage of pSOFA+AG compared to PELOD-2 in determining mortality in pediatric septic patients. It does this by first analyzing the association between predictors and mortality using logistic regression statistical tests and the Chi-square test.

CONCLUSION

The pediatric SOFA (pSOFA) score is more sensitive than the PELOD-2 score in assessing the mortality of pediatric septic patients. The use of the pSOFA+AG score was not more sensitive than the PELOD-2 score in assessing the mortality of pediatric septic patients.

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Conflict of interest

No conflict of interest has been declared in this study.

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Author contribution

Johaan Pawe Siampa conceptualized and designed the study, interpreted the data, and wrote the research manuscript. Arie Utariani and Elizeus Hanindito collected the data and checked the final result of the research.

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Original Research Report

CHONDROREGENERATIVE POTENTIAL OF PLATELET-RICH FIBRIN (PRF)-IMPREGNATED DECELLULARIZED BOVINE CARTILAGE SCAFFOLD IMPLANTED SUBCUTANEOUSLY

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ABSTRACT

The invention of alternative implants with regenerative potential comparable to autologous cartilage continues to be encouraged due to high morbidity of the donor site related to autologous harvesting process. This research attempted an invention of alternative implant using tissue engineering techniques in the form of endogenous regeneration by combining decellularized bovine cartilage scaffold with platelet-rich fibrin (BCPRF) that was implanted subcutaneously. The study aimed to compare the chondroregenerative potential between BCPRF and autologous cartilage in terms of the formation of newly-regenerated chondrocyte, the thickness of type II collagen produced, and the rate of cartilage resorption following the subcutaneous implantation. This study was conducted in a pretest-posttest control group design using New Zealand white rabbits. Forty-eight experimental samples were divided into two groups, then treated with subcutaneous implantation of BCPRF and autologous cartilage respectively. The results were evaluated after six weeks of implantation. Thirty-nine samples were evaluated. There was a significant difference found from both groups in terms of the formation of newly-regenerated chondrocyte, the thickness of type II collagen (p=0.000), and the implant resorption rate (p=0.000). The microscopic images demonstrated a superior chondroregenerative potential in the group receiving implantation of autologous cartilage compared to the group receiving BCPRF. The chondroregenerative potential for autologous cartilage and BCPRF differed significantly in terms of the formation of newly-regenerated chondrocyte, the deposition of type II collagen matrix, as well as the resorption rate.

***Keywords**: Autologous cartilage; platelet-rich fibrin; bovine scaffold; chondrocyte regeneration; medicine*

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Highlights:

1. This research compared the hondroregenerative potential betw een bovine cartilage scaffold w ith platelet-rich fibrin (BCPRF) and autologous cartilage.
2. The formation of new ly-regenerated chondrocyte, the thickness of type II collagen, and the rate of cartilage resorption follow ing the subcutaneous implantation w ere assessed.
3. BCPRF is highly biocompatible and can be developed as an alternative to alloplastic porous polyethylene (Medpor) implant material.

INTRODUCTION

The usage of cartilage grafts in corrective surgery for congenital craniofacial abnormalities, trauma, aesthetics, and tumor resection may be considered relatively high. Reconstruction using autologous cartilage is an oftenly used modality. However, autologous cartilage application slowly begins to be restricted due to some drawbacks in harvesting, such as donor-site morbidity, likelihood of dislocation, donor-site scarring, and risk of pneumothorax (Araco et al. 2006,Mischkow ski et al. 2008,G unter et al. 2008, Revell & Athanasiou 2009,Moon et al. 2012,Wee et al. 2015)

Autogenous cartilage graft is a biocompatible alternative available in the same surgical field (Soria-Gondek et al. 2022). Autologous cartilage is always the tissue of choice for transplantation purposes. Preserved or fresh homogenous cartilage graft is a valuable second choice. Heterogeneous cartilage from stingray or shark should only be considered if homogeneous

cartilage is not available There was good evidence that young cartilage grafts, with or without perichondrium, do not grow or increase in size after implantation in humans (Peer 1954). This encourages the discovery of other alternative modalities that can provide the same efficacy as autologous cartilage with minimal complications.

The new paradigm in tissue engineering nowadays has provided an opportunity to develop xenograft for tissue regeneration, especially cartilage. Cartilage tissue engineering has proven its effectiveness for cartilage regeneration. Xenografts have different properties, depending on their origin, nature, and processing. Bovine cartilage is a xenograft material that is often used as a scaffold due to its availability in nature. It is typically derived from cattles and pigs because of its osteoinductive and osteoconductive properties, low cost, and availability. However, it has shortcomings in immune response and carries the risk of transmitting animal diseases. Research have not yet reported significant differences in the use of animal-derived or synthetic biomaterials. Several lines of evidence suggest that synthetic materials have a lower risk of disease transmission (Barakat et al. 2008, Rhatomy et al. 2021). Deproteinization is an essential process to eliminate the antigenicity of xenograft bone. Effective strategies to eliminate the antigenicity of foreign bone are important in the development of xenogenic bone substitutes (Barakat et al. 2008).

Platelet-rich fibrin (PRF) is one of the sources of growth factors that has been widely studied lately. It is a fibrin matrix containing cytokines, growth factors, and cells that are progressively launched into wounds over time. Platelets are very vital for tissue healing (He et al. 2009). General traits of PRF consist of the amendment of centrifugation speed and time in addition to the dearth of anticoagulants/polymerization agents, differentiating it extensively from first-generation platelet concentrates or platelet-rich plasma (PRP). PRF differs from PRP because of its ability in providing the biochemical structure of a fibrin clot with platelets, cells, and circulating cytokines, and growth factors that have high affinity (Choukroun et al. 2006a, 2006b, Dohan Ehrenfest et al. 2009, Ghanaati et al. 2014). In addition, the discharge of growth factors is controlled and sustained owing to the fibrin scaffold, which can benefit the regeneration process (He et al. 2009, Dohan Ehrenfest et al. 2010, Pradeep et al. 2012, Kobayashi et al. 2016).

This research was conducted based on the tissue engineering-endogenous regeneration concept. The aim of this research was to examine whether the implant made from a combination of bovine cartilage and PRF (hereafter referred to as decellularized bovine cartilage scaffold-PRF implant (BCPRF)) has a potential comparable to autologous cartilage in regenerating cartilage tissue. In the future, the BCPRF can be developed as an alternative implant capable of replacing the use of autologous cartilage.

Rhatomy et al. (2021) stated that decellularized biomaterial scaffold limits the use of growth factor, resulting in better cost and resource efficiency. Organic materials are preferred because they have better biocompatibility and biodegradability than synthetic materials (Park & Cho 2010). Sponge bovine cartilage scaffold is a byproduct, which has no economic value and is usually discarded. This biomaterial is cheap and easy to acquire. Bovine cartilage scaffold will not damage the stem cell (Utomo & Rantam 2017, Mahyudin et al. 2018).

Tissue engineering technique requires three main components to regenerate a network, the cells, scaffolds, and growth factors (Vinatier et al. 2009). In terms of implant manufacturing technology, many studies combine these three components exogenously, with the aim of regenerating tissue in vivo (Utomo & Rantam 2017). In the endogenous regeneration concept, the potential of scaffold and growth factors combination is optimized exogenously, so it can endogenously stimulate the recruitment and differentiation of cells (e.g. endogenous mesenchymal stem cells/MSC). The result expected is in vivo regeneration of the tissue (Gulati & Poluri 2015). The advantage of this concept is that it does not require complicated procedure or large cost for exogenous stem cell preparation. In this study, the decellularized bovine cartilage scaffold proved to contain bone morphogenetic protein 4 (BMP4), which plays an important role in the differentiation of MSC towards chondrogenic lineage.

MATERIALS AND METHODS

Fabrication of BCPRF implant

Bovine articular cartilage obtained from the surface of cow joints was decellularized using 5% sodium dodecylsulfate (SDS) solution for 72 hours. To produce PRF, 10 ml of rabbit blood was centrifuged at 2700 rpm for 12 minutes. The gel-shaped middle layer was then extracted as PRF. The decellularized bovine cartilage was combined with the PRF through a mechanical mixing process with ratio of 5 g decellularized bovine cartilage and 1 mL PRF. Afterwards, the result went through the lyophilization process (freeze drying) for 2x24 hours. The outcome was a porous compact implant that was divided into 2x1x0.2 cm size (Figure 1).

Figure 1. Macroscopic appearance of BCPRF implants showing porous compact texture

Harvesting autologous cartilage

Autologous cartilage was obtained from rabbit auricle (2x1 cm in size, without its perichondrium layer), as seen in Figure 2. The dimension (length, width, and thickness) was then measured using a micrometer. The donor site was closed by primary intention using nylon.

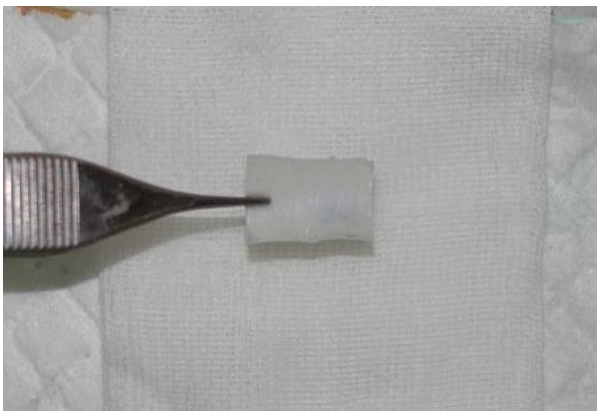

Figure 2. Autologous cartilage harvested from auricular region

Subcutaneous implantation

Two experimental groups, each consisted of 12 New Zealand male white rabbit (*Oryctolagus cuniculus)* weighed 3-3.5 kg, were prepared to undergo subcutaneous implantation using the BCPRF implant and autologous cartilage respectively. The number of samples for each group was 24 samples from 12 rabbits.

In the first group, BCPRF were implanted subcutaneously in the back area of the rabbits by creating a pocket. The implant sites were treated by primary intention using nylon. In the second group, autologous cartilages were implanted using the same method (Figure 3). Those implants were maintained for six weeks. Injection of 200 mg cefazoline was given intramuscularly once a day for three days after the implantation. The drop-out criteria included the emersion of signs of infection in the implant sites, implant extrusion, implant loss, and death of the rabbit.

Harvesting

After implantation, the implant and peri-implant tissue were then harvested from both experimental groups. The specimen volume was measured using VisiTrak for the length and width, while the thickness was measured histopathologically. The specimens were then preserved in 10% neutral-buffered formalin (NBF) solution.

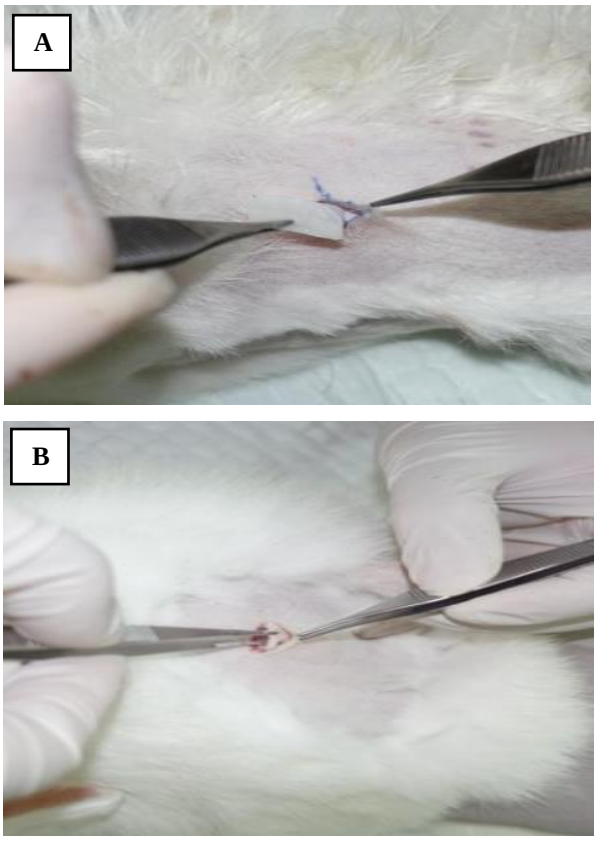

Figure 3. (A) subcutaneous implantation of autologous auricular cartilage; (B) similar process involving BCPRF implant

Evaluation and statistics

There are three parameters of chondroregenerative potential, i.e. the amount of chondrocyte formation, the thickness of type II collagen, and implant resorption rate, were assessed in a histopathological examination. The chondrocyte formation was counted after a hematoxylin-eosin staining from three fields of view

with 400x magnification. The thickness of type II collagen was measured by immunohistochemical staining with 100x magnification. The implant resorption rate was known by calculating the reduction/change in implant volume pre- and post-implantation. The results were then tested statistically using the Independent t-Test and Mann-Whitney U Test with 95% confidence interval (CI).

RESULTS

During 6 weeks of observation, the results yielded 2 dead rabbits, 2 implant exposures, and 2 infected implant sites in the autologous cartilage group, so the total evaluated samples was 16. The BCPRF group lost 1 implant, so the total evaluated samples became 23.

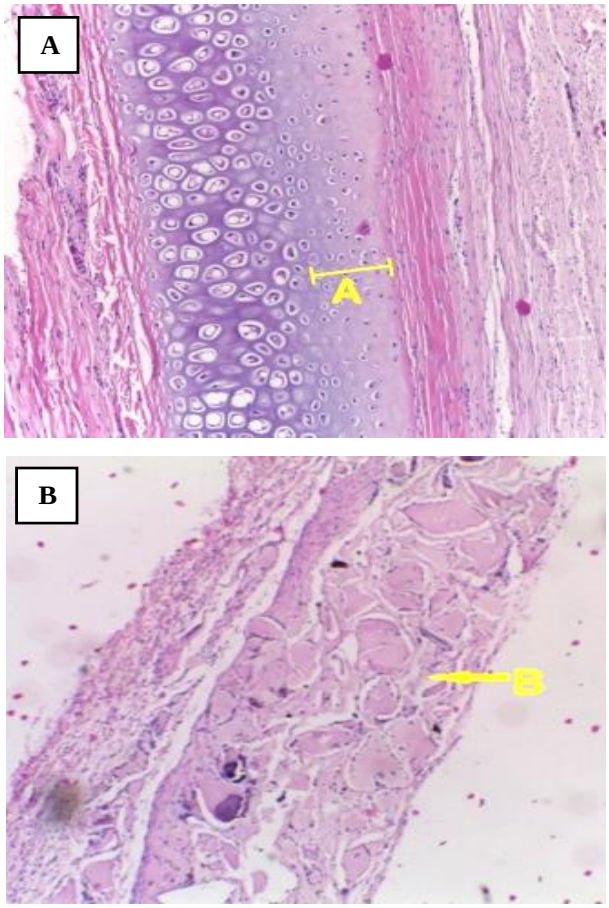

Figure 4. Hematoxyllin-eosin staining showing new chondrocyte formation in implanted autologous cartilage (A), compared to those none observed in BCPRF implantation (B)

The median of new chondrocytes from the peri-implant site was calculated from three fields of view with 400x magnification. The growth of new chondrocytes in the autologous cartilage group was 16.84±4.47 cells, meanwhile there was no growth of chondrocyte cells in the BCPRF group (Figure 4).

The thickness of type II collagen formed on the matrix between chondrocyte cells or peri-implant tissues in both groups was assessed using immunohistochemical staining with 100x magnification. In the autologous cartilage group, type II collagen thickness reached 23.05±7.59 μm. Whereas, in the BCPRF group, the thickness was 7.63±3.21 μm. Significant differences were found in the two groups (p=0.0000).

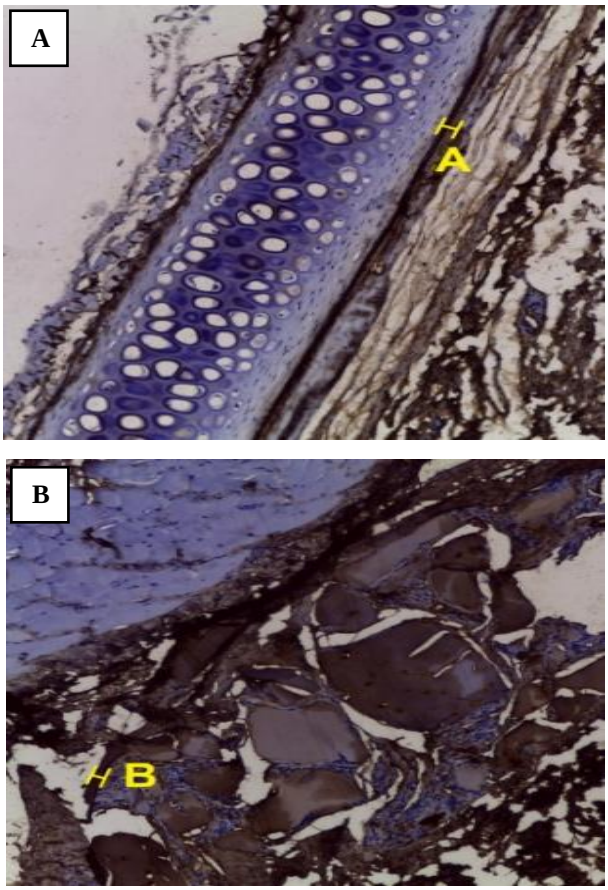

Figure 5. Immunohistochemical staining of type II collagen observed on peri-implant tissue of autologous cartilage (A) and BCPRF implant (B)

The implant resorption rate was measured by the percentage change in implant volume pre- and post-implantation. The volume was known by calculating the length, width, and thickness of the implant. In the autologous cartilage group, the percentage of resorption reached -6.94±12.86%. Whereas, in the BCPRF group, a reduction of -76.25±17.31% was obtained (Figure 6). Both were significantly different (p=0.0000).

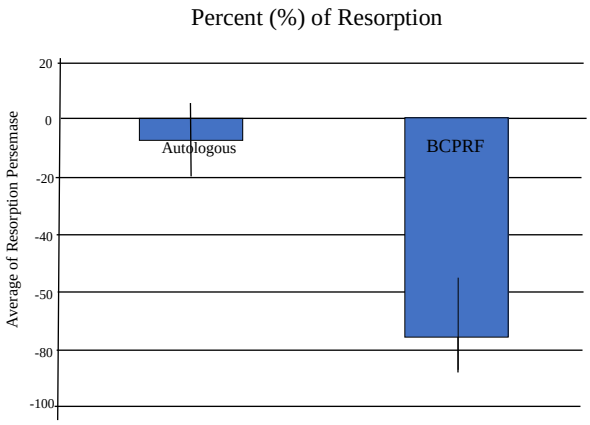

Figure 6. Rate of implant resorption comparing autologous cartilage implant (left bar) and BCPRF implant (right bar)

DISCUSSION

The results concluded that there was a significant difference in chondroregenerative capacity between autologous cartilage implant and BCPRF implant. The autologous cartilage implants proved to be superior in regenerating chondrocytes, producing chondroid matrix that was predominantly comprised of type II collagen, in addition to having lower implant resorption. In this study, autologous cartilage and BCPRF were implanted in a distant region that did not share equal characteristics with their native environments (Bimoseno et al. 2022). Subcutaneous tissue found on the back of rabbits is generally constituted of fibrocollagenous connective tissue and is devoid of cartilaginous tissue.

The ability of autologous cartilage implants to regenerate even outside of its native environment, as demonstrated in this study, may be attributed to the presence of active chondrocytes within the implants. The survival of autologous cartilage as graft stems from the process of plasma imbibition, which promotes chondrocytes to undergo a regenerative process termed as appositional growth. The recruitment of MSC to the wound of autologous cartilage implantation induces the differentiation of MSC towards the chondrocytic lineage due to an adequate differentiation signal. This signal is composed of active bone morphogenetic protein (BMP) and cartilage-derived morphogenetic protein (CDMP) molecules that are produced by active chondrocytes. The chondroid matrix found in autologous cartilage implants is optimal to direct the differentiation of MSC to form new cartilaginous structures. Adequate differentiation of MSC sustains the survival of autologous cartilage as a graft.

Complications found in this study, such as exposed (extruded) implants and infected autologous cartilage implant sites, might be caused by the fact that the skin pockets created were around the same size as the autologous cartilage implant, thus made them unable to accommodate the migration of the rigid autologous cartilage implant. This may have caused excessive surface tension of the skin suture, resulting in suture dehiscence. Surface tension may also generate inflammation and infection even though several studies claim that the use of autologous cartilage graft is relatively safer, with lower rates of infection and extrusion attributed to its minimal immune reaction (Wee et al. 2015).

The results of this study exhibited that autologous cartilage had greater regenerative potential than BCPRF implants when measured by the growth of chondrocytes, the production of type II collagen matrix, and the rate of resorption. The BCPRF implants demonstrated a relatively high rate of resorption or implant degradation, with an average volume post-implantation of 76%. In a study using polylactide (PLA) material, Odelius et al. (2011) suggested that pore size and porosity of implants are directly correlated with the degradation or resorption of implants. A highly porous implant is more likely to increase hydrolysis and degradation. Big pore size, porosity, and large network provide a good media for cell regeneration and the flow of nutrients into the scaffold (Loh & Choong 2013, Gariboldi & Best 2015).

The final process of BCPRF implant manufacturing is freeze-drying or lyophilization. This process produces microarchitecture that is dense and porous and contains relatively large pores. The high porosity of the microarchitecture allows for a greater surface area of the implant to undergo hydrolysis and degradation. This explains the high resorption and degradation rate observed in the BCPRF implants. The degradation process that occurred was hydrolytic, instead of immunologic or enzymatic. Therefore, the rates of inflammation, infection, and extrusion were lower in the BCPRF implants than the autologous cartilage. Optimal decellularization process allows the immunologic component of an implant to be omitted, thereby minimizing the degradation process that involves immune reactions.

The concept of tissue engineering triad combines the role of cells, scaffold, and morphogenic factor in the regeneration of tissue (Vinatier et al. 2009). According to the endogenous regeneration paradigm, BCPRF implants are designed to regenerate cartilaginous tissue by optimizing the recruitment of endogenous MSC into the injured/implanted area (Gulati & Poluri 2015). The endogenous MSC will differentiate to chondrogenic lineage via active morphogenetic proteins (CDMP1,

CDMP2, BMP2, BMP4, BMP6, and BMP9) that are found within the scaffold in the form of decellularized bovine cartilage matrix (Utomo & Rantam 2017). This differentiation signal is strengthened by supportive growth factors and BMP2 found in PRF. Thus, it is expected that a porous and chondroinductive scaffold media, due to its composition of morphogenic proteins and adequate growth factor signals from PRF, will influence the recruitment of MSC to the injured area and will differentiate it into mature chondrocytes.

The results of this study revealed that the regenerative capacity in the BCPRF implants, in terms of chondrocyte growth, was not proportionate to those found in the autologous cartilage implants. The lack of chondrocyte growth post-implantation of BCPRF might be attributed inadequate signals for morphogenic factors that direct the differentiation of MSC towards chondrogenesis. A study by Nakayama et al. (2003) explained that BMP4, a morphogenetic protein used during chondrogenesis, works in a dose-dependent manner. The study claimed that a greater dose of BMP4 (50 ng/mL) will stimulate greater production of cartilage. Conversely, a minimal dosage of BMP4 (5 ng/mL) is not sufficient to stimulate the formation of cartilage. This suggests that the inability of the BCPRF implants to stimulate chondrocyte production occured because of an inadequate number of morphogenetic proteins, thus resulting in a suboptimal MSC differentiation signal. This phenomenon might explain the inability of the implants to direct the differentiation of MSC. Unfortunately, this study did not evaluate the concentration of morphogenetic proteins contained within the BCPRF implants, in particular the CDMP1, CDMP2, BMP2, BMP4, BMP6, BMP9, and transforming growth factor beta (TGF-β).

The conditions explained above would influence the MSC to differentiate according to the more dominant differentiation signal, which would stimulate the formation of fibrocollagenous tissue. According to the histological examinations performed in this study, the fibrocollagenous tissue grew and invaded the porous structure of the BCPRF implants between the chondroid matrixes. The fibrocollagenous tissue was comprised of fibroblasts and collagen fibers that, upon immunohistochemical staining, also expressed type II collagen fibers. Nonetheless, the thickness of type II collagen of the fibrocollagenous tissue that grew in the BCPRF implants was not compared to the thickness of collagen produced by the autologous cartilage implants. The type II collagen matrixes that developed in the BCPRF implants were produced by fibrocollagenous tissue instead of mature chondrocytes. This study proposed that one of the parameters used to measure chondroregenerative capacity should be the production of type II collagen matrix produced by chondrocytes. On the other hand, the implantation of BCPRF implants did not result in the growth of chondrocytes.

It may be concluded that the regeneration of tissue requires a combination of cells, scaffold, and an adequate signal of morphogenetic proteins. The role of morphogenetic proteins has often been discussed in many studies regarding tissue engineering that involves implants, be it in vivo or in vitro (Li et al. 2015, Crecente-Campo et al. 2017, Lin et al. 2019).

Nevertheless, positive results were found in this study. The growth of fibrocollagenous tissue may be favorable with optimal integration of the BCPRF implant to its surrounding tissue. Though it lacks regenerative ability, the BCPRF may be developed as an implant material that relies on fibrocollagenous tissue formation (Utomo & Sari 2019, Utomo & Yusbida 2019). BCPRF has high biocompatibility because it is made from decellularized bovine cartilage matrix. This material may be developed as an alternative to alloplastic implant material, made of porous polyethylene (Medpor), that is widely used in reconstructive and aesthetic plastic surgery. The integration of alloplastic implants relies on their ability to facilitate fibrocollagenous tissue growth within the porous internal structure. The difference lies in the mechanical stability and resorption of the implants. Redesigning the manufacturing process of BCPRF implant can produce a strong and mechanically stable implant with low resorption rate. BCPRF implant may be developed into biomaterial that can compete with alloplastic implant material. The composition of its natural material may allow BCPRF implant to be superior to its alloplastic counterpart.

Strength and limitations

BMP4 is used in this study's scaffold made of bovine cartilage to help MSCs differentiate into chondrogenic lineages and based on the tissue engineering-endogenous regeneration concept. The development of BCPRF as a replacement implant that can replace the use of autologous cartilage is possible in the future.

CONCLUSION

The chondroregenerative potentials of autologous cartilage and BCPRF differ greatly with respect to the newly regenerated chondrocyte formation, type II collagen matrix deposition, and resorption rate. However, the BCPRF is made from decellularized bovine cartilage matrix, which makes it highly biocompatible. It can be developed as an alternative to alloplastic porous polyethylene (Medpor) implant material.

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Conflict of interest

There was no conflict of interest in this researchs.

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Author contribution

Putu Ardhy Parama Widyatmika conceptualized the study, wrote and prepared the original draft, and reviewed and edited the manuscript. Muhammad Sjaifuddin Noer developed the methodology and collected the resources. Magda Rosalina Hutagalung gave the validation of the study. All authors have read and agreed to the published version of the manuscript.

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Original Research Report

IDENTIFICATION OF PATHOGENIC BACTERIA IN FOOD SAMPLES FROM CAFETERIAS OF A UNIVERSITY IN DENPASAR, INDONESIA

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ABSTRACT

Foodborne diseases still remain a problem especially in public areas. This study aimed to identify pathogenic bacteria in foods sold at the cafeterias of Universitas Warmadewa, Denpasar, Indonesia. The presence of pathogenic bacteria in the food samples was identified using the brain heart infusion (BHI) agar for the growth of Escherichia coli *and* Streptococcus aureus*, selenite for* Shigella *and* Salmonella*, and alkaline peptone for* Vibrio cholerae*, then bacterial culture was conducted to identify the species. The results showed that* Escherichia coli *were found in two food samples, kangkong and jinggo rice, with the colony counts of 50-118.* Escherichia coli *was the most commonly found contaminant in food samples from the Warmadewa Cafeterias. Further suggestions must be offered to increase hygiene in the food processing by advising the stalls' owners to serve good foods and conducting regular inspections to assess the food quality.*

Keywords: *Pathogen; bacteria; food; consumption; public health*

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Highlights:

1. Eschericia colli was the most contaminant bacteria among food samples from Warmadewa Cafeterias
2. Further suggestions to increase the hygiene in food processing must be offered.

INTRODUCTION

Foodborne diseases are commonly found diseases around the world, which produce health problems through the distribution of infectious bacteria from food handlers (Adesiyun et al. 2020). As the name implies, foodborne diseases transmit through food (Akilan et al. 2020, Abolghait et al. 2020). Foodborne diseases can be caused by various types of microbes, such as coliforms and bacteria that most commonly cause food contaminations (i.e. *Escherichia coli* and *Salmonella* sp.) (Dallal et al. 2020). If *Escherichia coli* is present in water and food, then it indicates a contamination of the food and water (Ahmed & Shimamoto 2014, Adimasu et al. 2016). Bacteria often contaminate meat because of the high water and protein content that can foster bacterial growth (Ghoneim et al. 2020). As one of the most common public health problems, foodborne diseases commonly show clinical manifestation, such as nausea, vomiting, abdominal cramp, diarrhea, and fever (Jang et al. 2021).

Pathogenic bacteria have possibilities of contamination in any step of food processing (Sari et al. 2013). Therefore, food quality and sterile process as the important concepts of food handling must be implemented when selecting ingredients, storing and processing food, also serving and storing cooked food (Li et al. 2020). Hygiene and sanitation are efforts to avoid diseases. They are needed to protect food from contamination and disease-transmitting microorganisms (Atun 2016). They are also efforts to control the settings of kitchen, equipments used, food handlers, and ingredients that can prevent contaminated food from spreading diseases (Valero et al. 2016).

In developing countries, it is estimated that 70% of diarrhea cases are associated with the consumption of contaminated food (Aruna & Rajan 2017, El-Sharkawy

et al. 2017). Outbreaks of foodborne disease due to the contamination transmitted from food handlers are estimated at 30% (Sofiana 2012). In a research conducted at a school cafeteria in Central Jakarta area, 56.92% food samples and 52.89% beverage samples were positively contaminated by *E. coli* (Sofiana 2012). A previous research conducted in Java, Indonesia, showed that 25% of the food samples were contaminated with bacteria (Sunarno et al. 2010).

Food handlers and people managing places (especially public places) that sell foods and beverages should be given attention, so that they serve healthy and safe foods (Zhao et al. 2016). Schools and universities, such as Universitas Warmadewa, Denpasar, Indonesia, are public places that provide street foods. All cafeterias located in Universitas Warmadewa sell types of food that contain high water and protein content. Universitas Warmadewa has seven cafeterias that sell various types of foods and beverages. Data on knowledge and attitudes regarding hygiene and sanitation of the food handlers, who were actively selling foods, and the microbiological quality of foods in Universitas Warmadewa were not yet known. Based on the description above, it was necessary to conduct a research on the hygiene, sanitation, and microbiological quality of foods in Universitas Warmadewa.

MATERIALS AND METHODS

This study used a descriptive method with a cross-sectional approach. This research was conducted at all of the cafeterias of Universitas Warmadewa in July-December 2017. All of the food sellers acted as the respondents in this study. The samples used were all food handlers who played an active role in the cafeterias of Universitas Warmadewa and the foods sold at the cafeterias. The microbiological quality was assessed by identifying the presence of pathogenic bacteria in food samples obtained from the cafeterias of Universitas Warmadewa. The ethical compliance assessment result was obtained from the Research and Development Unit of Universitas Udayana, Denpasar, Indonesia, with the letter number 909/UN14.2.2/III/14LT/2018.

Following the methods of microbial detection and identification by (Ferone et al. 2020), the food samples were brought to the laboratory, then ground and wrapped in sterile containers. According to a study on evaluation of antimicrobial activity by (Grace et al. 2020), each of the samples should be weighed approximately five grams for foods and 10 mL for beverages, then taken directly using a dropper and put into the agar media to be incubated for 1x24 hours. The media used in this study were similar to what were used in a study by Happy et al. (2018). Brain heart infusion (BHI) agar was used for growing *Escherichia coli* and *Streptococcus aureus*, selenite medium for *Shigella* sp. and *Salmonella sp.*, and alkaline peptone for *Vibrio cholerae*. Food samples should be infused with the media, then preserved at 37^{o}C for 24 hours (Hussein et al. 2018, Yu et al. 2021). After a day, a dose of the enrichment media should be taken and then planted on the selective media, i.e. blood agar media for growing *Staphylococcus aureus*, MacConkey agar for growing *Escherichia coli*, *Salmonella-Shigella* (SS) agar for growing *Salmonella sp.* and *Shigella sp.*, and thiosulfate-citrate-bile-sucrose (TCBS) agar for growing *Vibrio cholerae* (Omara et al. 2017, Pei et al. 2020).

The next process after planting in the medium is carrying out the Gram staining and the indole, methyl red (MR), Voges-Proskauer (VP), and citrate (IMViC) (Tabashsum et al. 2013, Tarabees et al. 2017). In this research, the samples were taken to the Health Laboratory Unit (*Balai Laboratorium Kesehatan*), Bali Province, Indonesia, to check the bacteriological quality using bacterial culture. The bacteriological quality checks were carried out to determine the pathogenic bacteria contained in the foods and beverages sold at the cafeterias of Universitas Warmadewa. In a descriptive study, the researcher collects data and analyzes the data descriptively (Zhao et al. 2016). The data from this research were also collected and then analyzed in the same manner.

RESULTS

Two to three food and beverage samples were taken from seven cafeterias of Universitas Warmadewa. The samples studied were 22 samples, with 15 food specimens and 7 beverage specimens. The sampling was carried out in the morning before the foods were sold out. *Escherichia coli* is an indicator of food sanitation and food processing quality. Two foods were found to be contaminated by *Escherichia coli*, i.e. kangkong and jinggo rice. The colony counts of *Escherichia coli* in the kangkong and jinggo rice were 50 and 118 respectively. However, the regulation of the Indonesian Ministry of Health number 1096/Menkes/Per/VI/2011 concerning the requirements for sanitation and hygiene of catering services accredited to ISO/IEC 17025:2005 states that the presence of *Escherichia coli* bacteria in foods and beverages should be 0 gr/mL. Test result of >0 indicate that the food is not allowed to be consumed. It also indicates that the food is not processed well by the food handler.

The only pathogenic microbes found in the foods sold at the cafeterias of Universitas Warmadewa were *Escherichia coli*, while the identification of the other bacteria came out as negative as listed in Table 1.

Table 1. Identification of pathogenic bacteria in foods and beverages from the cafeterias of Universitas Warmadewa

No	Food sample	Colony count				
		E. coli	*Staphylococcus aureus*	*Salmonella*	*Shigella*	*Vibrio cholerae*
1	Kangkong	50 (+)	(-)	(-)	(-)	(-)
2	Fried chicken and lalapan	0	(-)	(-)	(-)	(-)
3	Tempe and lalapan	0	(-)	(-)	(-)	(-)
4	Saltwater fish	0	(-)	(-)	(-)	(-)
5	Betutu chicken	0	(-)	(-)	(-)	(-)
6	Jinggo rice	118 (+)	(-)	(-)	(-)	(-)
7	Tipat tahu	0	(-)	(-)	(-)	(-)
8	Stir fried tempe and tomato	0	(-)	(-)	(-)	(-)
9	Sayur jepang	0	(-)	(-)	(-)	(-)
10	Fried rice	0	(-)	(-)	(-)	(-)
11	Beef rendang	0	(-)	(-)	(-)	(-)
12	Sayur urap	0	(-)	(-)	(-)	(-)
13	Chicken soto	0	(-)	(-)	(-)	(-)
14	Tipat cantok	0	(-)	(-)	(-)	(-)
15	Rujak	0	(-)	(-)	(-)	(-)
16	Mango juice	0	(-)	(-)	(-)	(-)
17	Iced tea 1	0	(-)	(-)	(-)	(-)
18	Iced orange squash 1	0	(-)	(-)	(-)	(-)
19	Iced tea 2	0	(-)	(-)	(-)	(-)
20	Iced tea 3	0	(-)	(-)	(-)	(-)
21	Iced sugar water	0	(-)	(-)	(-)	(-)
22	Iced orange squash 2	0	(-)	(-)	(-)	(-)

DISCUSSION

According to the literature review, *Escherichia coli* passes on to humans from feces. When a person performs defecation activities, they might not wash their hands thoroughly with enough soap and tap water. It contributes to the findings of *Escherichia coli* that can transfer to human hands (Zhao et al. 2016). The presence of *Escherichia coli* in foods or beverages correlate with the spreading of pathogens that can cause gastrointestinal problems, such as diarrhea and toxication or poisoning (Ghoneim et al. 2020).

The results of the observations showed that only one cafeteria in Universitas Warmadewa where the food handlers used utensils when taking foods in the serving and storing processes. Whereas, the food handlers in the other cafeterias used bare hands without gloves and utensils. They only occasionally washed their hands before and after taking foods. There were only five food handlers at the cafeterias of Universitas Warmadewa who used aprons, while none used head coverings when processing and serving foods. Based on the results of the questionnaires distributed before sampling, 100% respondents answered correctly regarding knowledge of food hygiene and sanitation. The attitude of the food handlers mostly agreed with the requirements of food hygiene and sanitation.

Strength and limitation

This study was done in one cafeteria at Warmadewa University so that future studies may also be done there. The food handlers' attitudes were generally in line with Universitas Warmadewa's and other universities' standards for food hygiene and sanitation.

CONCLUSION

Eschericia coli is the most commonly found contaminant bacteria in food samples from the cafeterias of Universitas Warmadewa. Futher suggestions must be offered to increase the hygiene in food processing by guiding the stalls' owners to serve good foods and conducting regular inspections to assess the food quality.

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Conflict of interest

None declared.

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Author Contribution

The list of author contribution is as follows. AAA LP collected and provided the samples, then transferred them to the Health Laboratory Unit (*Balai Laboratorium Kesehatan*), Bali Province, Indonesia. PIB wrote the manuscript and analyzed the data. PAS performed the culture of the samples, then tabulated the results. NWW supported us by gathering the food handlers who allowed us to take their foods as the research samples.

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Original Research Report

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND IN VITRO ANTIPLASMODIAL ACTIVITY OF *Terminalia mantaly* AGAINST *Plasmodium falciparum*

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ABSTRACT

Malaria has been one of the world's worst killer diseases throughout recorded human history. Despite attempts to eradicate the disease, it remains a global burden. This could be a result of parasite resistance to current therapy. However, this research aimed at evaluating the in vitro antimalarial activity of ethanolic extracts of Terminalia mantaly *on* Plasmodium falciparum. *The plant extracts were prepared by cold maceration in 70% ethanol and air-dried by a rotary evaporator. The phytochemical analysis was carried out using standard procedures outlined in the Analytical methods of the Association of Official Analytical Chemists (AOAC, 1990) which indicates the presence of tannins, alkaloids, saponins, flavonoids, glycosides, phenol, steroids, and balsam. The in vitro antimalarial assay was carried out according to the method described by WHO (2001). All data were represented as Mean ± Standard deviation. Ethanolic extracts of the three parts of the plant were subjected to in vitro antimalarial activity at three concentrations (300 mg, 150 mg, and 75 mg) in four replicates with artemether (standard drug) as a positive control. Stem bark at 300 mg/kg completely cleared the parasites with a 0.00% parasitaemia rate and there was no significant difference when compared with positive control at p<0.005 value of 1.00. This study affirms the use of the plant for the treatment of malaria.*

Keywords: *Malaria; terminalia mantaly; plasmodium falciparum; tropical disease*

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Highlights:

1. Malaria is prevalent in many populations of communities despite preventive measures.
2. The experimental was screened for bioactive components which could be the reason for the antimalarial effect and the plant shows dose dependent antimalarial activity.

INTRODUCTION

Malaria has been one of the world's worst killer diseases throughout recorded human history. Malaria is a life-threatening disease caused by protozoa of the genus *Plasmodium* transmitted by the female anopheles mosquito (WHO,2019). Five species of *plasmodium* are known to cause malaria in man: *P. vivax*, *P. falciparum*, *P. malariae*, *P. ovale*, and *Plasmodium knowlesi* (Abdulrazaq et al. 2020).

Despite attempts to eradicate malaria, it remains one of the worst diseases in terms of deaths annually and has actually increased in incidence since the 1970s (Taylor et al. 2006). According to the World malaria report released in December 2019, there were 228 million cases of malaria in 2018, and the estimated number of malaria deaths stood at 405,000 (WHO. 2019). In 2017, five countries accounted for nearly half of all malaria cases worldwide: Nigeria (25%), the Democratic Republic of the Congo (11%), Mozambique (5%), India (4%), and Uganda (4%) (WHO, 2019). Children under 5 years of age are the most vulnerable group affected by malaria; in 2017, they accounted for 61% (266,000) of all malaria deaths worldwide and this could be the result of low immunity.

Nigeria is one of the nations with the highest rates of malaria-related morbidity and mortality. A recent analysis of malaria risk and mortality in Nigeria revealed that, despite a global decline in malaria rates, cases there have climbed exponentially, killing many people (Kayode and Godwin, 2017; Samuel B and Adekunle YA, 2021). The World Health Organization

has estimated the malaria mortality rate for children under five in Nigeria at 729 per 100,000. About 300-500 million clinical cases are observed and 1.5–2.7 million deaths occur each year due to malaria, most of whom are children and pregnant women (Otubanjo 2013). Globally, malaria deaths have declined by 10% since 2000 and many African countries have achieved a 50% reduction in malaria interventions (Otubanjo. 2013).

Currently, four artemisinin combination therapies (ACTs) are recommended for the treatment of malaria: Artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine and artesunate–sulfadoxine-pyrimethamine (WHO. 2009). ACTs are often safe and effective, however there are still differences in their performance in areas where malaria is endemic (Hodel et al. 2013). Studies have revealed that the variability and susceptibility of malaria are influenced by human genetic variables (Driss et al. 2011). It similarly influences how medications are metabolized and how readily they are absorbed, which has an impact on how effective pharmacological therapy is (Desta and Flockhart, 2017). However, malaria parasites develop resistance to most of the available and affordable antimalarial drugs (Teka et al. 2020). Antimalarial drug development and discovery is expected to provide new drugs that not only have antimalarial activity in vitro and in vivo, but also have safety mechanisms applicableto humans (Ekasari, W et al. 2021).

Parasite resistance has caused some of the least expensive traditional antimalarial drugs to be ineffective. As such, there is an urgent need for new antimalarial therapies (Bekono et al. 2020). With an estimated protective efficacy for routine use, effective management of severe malaria patients has a great potential to reduce case fatality from malaria (Thwing J et al. 2011). Effective therapy can also stop consequences including developmental impairment (Carter JA et al. 2005), infection recurrence, and eventually, the establishment and spread of parasites that are resistant to treatment (WHO. 2021).

In the sub-Saharan African region, hundreds of plants are traditionally used for the treatment of malaria (Teka et al. 2020). In order to identify the bioactive components present in medicinal plants used in conventional medicine, phytochemical screening is a crucial step (Afnan Alqethami et al. 2021). Therefore, phytochemical screenings of medicinal plants for bioactive antimalarial compounds will help in the development of new antimalarial drugs to which the parasites are not resistant. Some studies had been conducted to show the efficacies of plants in the treatment of malaria through phytochemical screening of their constituents globally (Ekasari et al. 2021, Kurniawan et al. 2020). A variety of diseases are treated using plants because they have antibacterial properties. The natural ingredients used in these early attempts—typically native plants or their extracts—were successful in many cases. Green plants have the widest range of synthetic activity and are the source of numerous beneficial chemicals. Coincidentally, over the past ten years, there have also been an increasing number of in-depth research on extracts and biologically active chemicals obtained from plant species used in herbal medicine or natural therapies. *Terminalia mantaly* is a plant used in herbal medicine. (Ebele OP et al. 2021).

Terminalia mantaly is also called umbrella tree. It is a plant of the family of Combretaceae used in traditional medicinal practice in Madagascar, where its stem bark and leaves are used for the treatment of dysentery, mouth candidiasis, and postpartum care. There are almost 300 species of huge trees in the genus *Terminalia*, which is found across tropical areas of the world and belongs to the flowering plant family Combretaceae. Malaria is only one of the many ailments or symptoms that *Terminalia mantaly* are frequently used to treat by traditional healers (Titanji et al. 2008; Ngouana et al. 2015). In Côte d'Ivoire, the leaves are used in the treatment of malaria. *Terminalia mantaly* is a plant of the Cameroonian pharmacopeia used for malaria and/or related symptoms (Tali et al. 2020).

MATERIALS AND METHODS

Fresh plant of *Terminalia mantaly* H. Perrier was collected from the premises of Kebbi State University of Science and Technology Aliero around March 2019 and was authenticated at the herbarium in the Department of Plant Science and Biotechnology in Kebbi State University of Science and Technology Aliero, Kebbi State, Nigeria with voucher No-315.

The fresh leaves, roots, and stem bark of *Terminalia mantaly* were shade dried in the laboratory at room temperature separately. The dried leaves, roots, and stem bark were pounded to powder using mortar separately. Each sample was cold macerated in 70% ethanol at room temperature for 72 hours and then filtered using a muslin cloth. The filtrates were dried in a rotary evaporator at 70^{o}C. The extracts were stored in the freezer until required for use.

Plasmodium falciparum was obtained from the General laboratory in Sir Yahaya Memorial Hospital Birnin Kebbi, Kebbi State, Nigeria, and was taken to Zoology laboratory in Kebbi State University of Science and Technology Aliero, Kebbi State, Nigeria.

The phytochemical analysis was carried out using standard procedures outlined in the Analytical methods of the Association of Official Analytical Chemists (AOAC 1990) to screen the presence of bioactive compounds: tannins, alkaloids, saponins, flavonoids, glycosides, phenol, steroids, and balsam.

This was carried out according to the technique described by Trager and Jensen (1976). A packet of Rosewell Memorial pack Institute (RPMI) 1640 medium (containing 25 mM of HEPES buffer, glucose) was dissolved in 960 ml of distilled water, and 40 µg/ml of gentamycin sulfate (1.2 ml of Gentamycin/L) was added. It was passed through a millipore filter of 0.22 µm porosity and was stored at 4ºC in 96 ml aliquots in a glass media bottle. Exactly 4.2 ml of 5% sodium bicarbonate (5 gms of sodium bicarbonate dissolved in 100 ml double distilled water and filtered through a millipore filter of 0.22 µm porosity and stored at 4°C) was added to 96 ml of stock RPMI 1640 media (incomplete media). O+ blood was collected in a centrifuge tube without anticoagulant and kept at 4°C. It was centrifuged at 10000 x g for 20 min at 4°C the next day. Serum collected was separated aseptically and kept in aliquots. The serum was inactivated by using a water bath at 56°C for half an hour. Normal inactivated O+ human serum (10 ml) was added to 90 ml of incomplete media to make complete malaria media (CMM).

Uninfected cells were added to 0.75 % of parasitaemia and diluted with CMM to get 0.5 % cell suspension (5 % hematocrit). The culture was kept in a candle jar in an atmosphere of CO_2 at 37°C for 48 hours. After every 48 hours the media was removed using a sterile Pasteur pipette without disturbing the cells that settled down. Then the cells mixed without frothing and a drop of blood was placed on the slide to make a thin film. The prepared thin film was stained and examined for parasitaemia.

This was done in 96 well plates according to the method of WHO (In-vitro micro test (Mark, III) by assessing the maturation of the schizonts. Rosewell Memorial pack Institute (RPMI) 1640 was incorporated. Dilution was prepared from the extracts of leaves, roots and stem bark of *Terminalia mantaly* at concentrations of 300 mg, 150 mg and 75 mg in four groups each. Positive controls were treated with 1 ml Arthemether injection and negative controls were untreated. 200 microliters from blood mixture with media was added to each well in the plate and was incubated for 48 hours in a CO_2 incubator at 37ºC. The samples were harvested and smeared on slides. The blood films were fixed with methanol, stained with Geimsa at pH 7.2 for 10 minutes and examined under the microscope for the presence of parasites. The parasite density was calculated for each well in the plate by comparing the parasitaemia in each group. The antimalarial activity of the plant extracts at different concentrations were recorded. Percentage parasitaemia was obtained using the formula below. At each concentration of the extract, number of parasites were counted in quadrant, their mean and standard deviation were recorded and percentage parasitaemia was calculated using the formula below (Hagazy et al. 2020).

$$\% \text{ parasitaemia} = \frac{\text{Number of Parasitized cells}}{\text{Total Number of cells}} X100$$

RESULTS

Phytochemical screening of *Terminalia mantaly*

The phytochemical screening revealed the presence of tannins, alkaloids, saponins, flavonoids, glycosides, steroids, phenols and balsam, whereas terpenes are completely absent.

The composition of tannins in stem bark and leaves is much, whereas little in roots. Alkaloids and phenols are little in both three parts of the plant. The composition of saponins is very much in stem bark, whereas much in leaves and little in roots. Flavonoids are much in stem bark but little in leaves and roots. The composition of glycosides, steroids and balsam are much in all the three parts of the plant but terpenes are completely absent in all the parts of the experimental plant.

Table 1. The phytochemical screening of *Terminalia mantaly*

Phytochemicals	Stem bark	Leaves	Roots
Tannins	++	++	+
Alkaloids	+	+	+
Saponins	+++	++	+
Flavonoids	++	+	+
Glycosides	++	++	++
Steroids	++	++	++
Phenols	+	+	+
Balsam	++	++	++
Terpenes	-	-	-

KEY: +++= Very much, ++= Much, += Little, -=Absent

The in vitro antimalarial potential of the ethanolic extracts of roots, leaves, and stem bark of *Terminalia mantaly* on *Plasmodium falciparum* after 24 hours of incubation

The result of in vitro antimalarial activity of the ethanolic extract of roots, leaves and stem bark of *Terminalia mantaly* on *Plasmodium falciparum* after 24 hours of incubation are presented in Table 2. The stem bark ethanol extracts of the plant were recorded as follows: parasitaemia rate at 300 mg was 2.04% (3.50±4.43), parasitaemia rate at 150 mg was 23.32% (40.00±7.07), and parasitaemia rate at 75 mg was 30.76% (52.75±10.68). For positive control, the parasitaemia rate recorded after 24 hours of incubation was 1.17% (2.00±2.82).

The leaves ethanol extracts of the plant recorded after 24 hours were as follows: parasitaemia rate at 300 mg was 16.67% (53.75±65.04), parasitaemia rate at 150 mg was 26.12% (84.25±60.84), and parasitaemia rate at 75 mg was 47.36% (1.52±66.30), while the parasitaemia rate for positive control was 4.34% (14.00±23.33). The roots ethanol extracts of the plant recorded after 24 hours were as follows: parasitaemia rate at 300 mg was 23.67% (60.20±62.82), parasitaemia rate at 150 mg was 35.46% (90.75±49.21), parasitaemia rate at 75 mg was 64.44% (1.64±65.09), and parasitaemia rate for positive control was 1.57% (4.00 ±2.00) (Table 2).

Table 2. The in vitro antimalarial potential of the ethanolic extracts of roots, leaves, and stem bark of *Terminalia mantaly* on *Plasmodium falciparum* after 24 hours of incubation

Concentration	(Mean ± SD)					
	Stem bark		Leaves		Roots	
	Parasite count	Parasitaemia %	Parasite count	Parasitaemia %	Parasite count	Parasitaemia %
Positive Ctrl	2.00 ± 2.82[a]	1.17%	14.00 ± 23.33[a]	4.34%	4.00 ± 2.00[a]	1.57%
300 mg	3.50 ± 4.43[a]	2.04%	53.75 ± 65.04[a]	16.67%	60.25 ± 62.82[ab]	23.67%
150 mg	40.00 ± 7.07[b]	23.32%	84.25 ± 60.84[ab]	26.12%	90.75 ± 49.21[bc]	35.46%
75 mg	52.75 ± 10.68[c]	30.76%	1.52 ± 66.30[b]	47.36%	1.64 ± 65.09[c]	64.44%

KEY: SD= standard deviation, Superscripts= means followed by the same superscripts are statistically the same with the control using one-way ANOVA post hoc (Duncan) at p< 0.05, others had statistically significant lower antimalarial activity with the control (Positive).

Table 3. The *In-vitro* antimalarial potential of the ethanolic extracts of roots, leaves, and stem bark of *Terminalia mantaly* on *Plasmodium falciparum* after 48 hours of incubation

Concentration	(Mean ± SD)					
	Stem bark		Leaves		Roots	
	Parasite count	Parasitaemia %	Parasite count	Parasitaemia %	Parasite count	Parasitaemia %
Positive Ctrl	0.00 ± 0.00[a]	0.00%	2.75 ± 4.85[a]	0.57%	0.75 ± 0.95[a]	0.08%
300 mg	0.00 ± 0.00[a]	0.00%	5.50 ± 7.14[a]	1.15%	50.25 ± 62.99[ab]	16.48%
150 mg	1.50 ± 1.29[c]	0.87%	24.00 ± 18.18[a]	5.00%	74.50 ± 52.00[ab]	24.45%
75 mg	2.50 ± 1.29[c]	1.45%	57.0 ± 35.18[b]	11.88%	1.10 ± 48.09[c]	36.23%

KEY: SD= standard deviation, Superscripts= means followed by the same superscripts are statistically the same with the control using one-way ANOVA post hoc (Duncan) at p< 0.05, others had statistically significant lower antimalarial activity with the control (Positive).

The in vitro antimalarial potential of the ethanolic extracts of roots, leaves, and stem bark of *Terminalia mantaly* on *Plasmodium falciparum* after 48 hours of incubation.

The result of in vitro antimalarial activity of the ethanolic extract of roots, leaves and stem bark of *Terminalia mantaly* on *Plasmodium falciparum* after 48 hours of incubation are presented in Table 3.

The stem bark ethanol extracts of the plant after 48 hours of incubation at 300 mg were recorded: parasitaemia rate at 300 mg was 0.00% (0.00±0.00), parasitaemia rate at 150 mg was 0.87% (1.50±1.29), parasitaemia rate at 75 mg was 1.45% (2.50±1.29), and parasitaemia rate for positive control was 0.00% (0.00±0.00).

The leaves ethanol extracts of the plant at 300 mg were recorded 1.15% (5.50±7.14) for parasitaemia rate after 48 hours of incubation, 5.00% (24.04±18.18) for parasitaemia rate at 150 mg, 11.88% (57.00±35.18) for parasitaemia rate at 75 mg, and 0.57% (2.75±4.85) for positive control. The roots ethanol extracts of the plant at 300 mg were recorded 16.48% (50.25±62.99) for parasitaemia rate after 48 hours of incubation, 24.45% (74.50±52.00) for parasitaemia rate at 150 mg, 36.23% (1.10±48.09) for parasitaemia rate at 75 mg, and 0.08% (0.75±0.95) for positive control.

DISCUSSION

The phytochemical screening of the extracts showed the presence of tannins, alkaloids, saponins, flavonoids, glycosides, phenol, steroids and balsam. It agreed with the findings of (Tali et al. 2020) and (Mbouna et al. 2018). However, glycosides and balsam were not detected. It could be a result of genetic and environmental factors as they influenced the content and composition of secondary metabolites in plants. Similarly, these findings were in line with the findings of Mudi and Muhammad (2009). However, phenols were not detected and this could be a result of species differences as they worked with *Terminalia catappa*. Similarly, this work was in agreement with the work of Emilie et al. (2015), but tannins were not detected. Biological activity is attributed to the presence of various secondary metabolites in plants. Alkaloids, flavonoids, and saponins are the active constituents with an antimalarial activity which reduced parasitaemia in extract-treated groups in vitro antiplasmodial activity and *Plasmodium berghei* infected rats, hence prolonged the life span of the rats in the experiment. The quantity of phytochemical constituents of the plant determines the extent of its bioactivity. Likewise, the presence of more than one secondary metabolite in a plant determines the extent of its bioactivity as reported by (Musila *et al*. 2013). Alkaloids are the major classes of compounds with antimalarial activity and are detected in three parts of the plant. Alkaloids, steroids, and

saponins have been reported to be detrimental to several infectious protozoans such as *Plasmodium falciparum* and these bioactive compounds have been reported to have many medicinal purposes and play a vital role in the antimalarial activity.

The result of in vitro antimalarial activity showed that the stem bark ethanolic extract of *Terminalia mantaly* had the highest antimalarial activity on *Plasmodium falciparum*. Stem bark completely cleared the parasites when treated with 300 mg of the extract after 48 hours of incubation and there was no statistically significant difference of $p<0.05$ in their result when compared with the positive control. The antimalarial activity of stem bark aqueous extract was reported by (Tali et al. 2020). It was in agreement with the findings in this study as they found out that the stem bark of *Terminalia mantaly* presented the highest antiplasmodial activities in vitro on both resistant and sensitive strains of *P. falciparum* with IC50*Pf*W2 = 0.809 µg/ml. Similarly, Anti-*Plasmodium falciparum* activity of extracts from 10 Cameroonian medicinal plants was reported by Marie et al. (2018). It agreed with these findings as they reported that the highest SI values were obtained for the decoction extract of leaves and stem bark of *Terminalia mantaly* (SI>80.32). Decoction extracts of leaves of *Terminalia catappa* and *Terminalia mantaly* were considered of interest since they displayed high antiplasmodial activity (IC50 = 1.90–8.10 µg/mL) with high selectivity indices (SI > 31.20) against both *Plasmodium falciparum* 3D7 and INDO strains. The findings confirmed the use of many of these plants in the treatment of malaria and related symptoms.

Strength and limitation

The study propeses a treatment strategy that utilizes locally available and afffordable materials. It emphasizes the significance of inexpence and readily available malaria treatment, as this disease is frequent in low- and middle-income countries. clinical trials are still necessary to demostrate the efficacy of *Terminalia mantaly* ethanolic extract as a malaria treatment.

CONCLUSION

Phytochemical screening revealed the presence of some secondary metabolites at different concentrations in the three parts of the plant. Alkaloids, flavonoids, and saponins were the active constituents that could be the reason for the antimalarial activity on *Plasmodium falciparum* as they were reported to be the secondary metabolites responsible for antimalarial activity.

All the plant part extracts in vitro analysis showed antimalarial activity on *Plasmodium falciparum* on different doses of the plant extract and this study showed that the extract from the stem bark has great potential to cure malaria as it clears the malaria parasites after 48 hours of incubation in a CO_2 incubator. More research is recommended on the use of *Terminalia mantaly* to treat other diseases in order to develop new drugs for the treatment of a variety of illnesses to which the parasites are not resistant in order to reduce the global burden.

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Conflict of interest

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Author contribution

BMU wrote, verified the analytical methods, and revise the manuscript from reviewer, DDA and DYK checked the sources and conceived the original manuscript.

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Original Research Report

THE INFLUENCE OF SAFETY COMMUNICATIONS AND SAFETY PROMOTION POLICIES ON SAFETY PERFORMANCE AMONG NURSES IN THE EMERGENCY DEPARTMENT AT A TERTIARY HOSPITAL IN SURABAYA, INDONESIA

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ABSTRACT

The application of the occupational safety and health principles in work activities is expected to reduce the occurrence of work-related accidents. This research was conducted in the Emergency Department of a tertiary hospital of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, in 2019 and aimed to determine the influence of safety communications and safety promotion policies on the safety performance among nurses. This research was a quantitative research with a cross-sectional design study. The population in this study were 208 emergency room nurses at Dr. Soetomo General Academic Hospital with a total sample of 68 people using a simple random sampling technique. The results showed that the correlation between safety communications variable with safety performance had a p-value of 0.035 (<0.05) and the correlation between safety promotion policies variable with safety performance had a p-value of 0.319 (>0.05), indicating that there was an influence of safety communications on safety performance, while there was no influence of safety promotion policies on safety performance among nurses in the Emergency Department of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Keywords: *Safety communications*; *safety promotion policies*; *safety performance; public health*

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Highlights:

1. To reduce the occurrence of work-related accidents, the application of occupational safety and health principles in work activities is necessary.
2. This research analyzed the safety communications and safety promotion policies on the safety performance among nurses.
3. Safety communications have influence on safety performance, while safety promotion policies have no influence on safety performance.

INTRODUCTION

Hospital workers, such as health workers, laboratory workers, housekeeping workers, central sterile supply department (CSSD) workers, or even garbage collectors, have a great risk of getting healthcare associated infections (HAIs) or work-related infections. The implementation of and commitment to occupational and environmental health and safety are some of the efforts to protect all levels of hospital society and the environment from potential hazards caused by hospital activities. A study from Aghaei et al. (2020) showed that the most common occupational accidents in healthcare units are needle stick injuries. According to a research from Mayangkara et al. (2021), even with the implementation of occupational health and safety (OSH) in a hospital, minimizing occupational accidents is difficult due to some sectoral regulations of OSH from various agencies.

A good company is a company that implements optimal safety programs for the employees because they aware that it is directly related to their productivity and able to motivate them to improve their work quality (Purnomo et al. 2018). Verra et al. (2019) found that most establishments in European Union countries take preventive measures on direct physical harm. It shows that European Union establishments have great concern about safety. But, it was found that only 29.5% of them

take measures to promote health. Based on an integrative literature review from Wagner et al. (2020), the determinants of occupational safety culture in hospitals and other workplaces mostly include management and colleagues, the characteristics and circumstances of workplace, and the characteristics of employees. In a sustainability report, frequently raised issues of concern are the efforts to ensure the safety of employees in some industries (Wang et al. 2020).

Sustainable Development Goals (SDGs) are commitment to efforts in improving community welfare nationally and globally. Health and welfare are two variables that bind and influence each other in every citizen. Therefore, the state must ensure access to health for all citizens and make preventive efforts so that the community is not exposed to various infectious diseases (Irhamsyah 2020). A company that has been certified in quality, environmental, and occupational health and safety management has an important role in the success of the SDGs in three sustainability dimensions (economic, environmental, and social) (Fonseca & Carvalho 2019).

Based on most studies, in the effective implementation of safety management, there is safety performance as the major compelling force (Ajmal et al. 2021). Occupational safety has a crucial role to enhance safety behavior as well as safety performance in the workplace (Asamani 2020). A hierarchical framework from a literature review by Mohammadi et al. (2018) explains that in order to determine the safety performance, not only management activities within project levels, but interactions among factors at different levels are also needed. It shows that each hierarchical level has an important role in determining the safety performance. Safety management practices are management practices, roles, and functions designed by companies to improve employee safety, which consists of 6 dimensions, i.e. management commitment related to safety, safety training, worker involvement in solving safety problems, safety communications, safety rules and procedures, and safety promotion policies. Safety communication is an activity to convey safety information to workers (Vinodkumar & Bhasi 2010). Safety communication can be measured using indicators from Vinodkumar & Bhasi (2010), which include discussing safety issues with leaders, discussing safety issues in meetings, and accessing safety informations. A research conducted by Supriyanto & Anggraini (2020) showed that communication has a significant influence on employee performance. A research by Ulva (2017) studied communication procedures to improve effective communication using the situation, background, assessment, and recommendation (SBAR) and write, read, and confirm (WRC) systems at a hospital in Padang, although in practice there are still problems in the confirmation sheet that have not been completely available. Moreover, teamwork and communication improvement training are necessary to promote safety culture and it is correlated with increased patient safety and fewer adverse events (Fukami et al. 2020). The safety promotion policies are efforts to motivate the workforce to prioritize safety when working. Safety promotion policies can be measured using indicators of the existence of a safety incentive program. An organization's perceptions can be the key to obtain support from the managerial roles about the implementation of health promoting policies and programs, so it can be implemented properly (Biswas et al. 2021). By implementing safety promotion policies, it reflects the good management commitment and signifies the proactive attitude toward safety (Subramaniam 2016).

Moreover, Linnan et al. (2019) found that in the United States, an OSH and workplace health promotion expert need to be part of multi-disciplinary teams within the State and Territorial Health Departments to bring critical worker health perspectives to the public health efforts.

Dr. Soetomo General Academic Hospital, Surabaya, is one of tertiary hospitals in East Java with type A category. Based on data obtained from the Occupational Health and Safety Committee of the hospital, there were 18 incidents related to OHS in 2017 and 13 incidents of work-related accidents in 2018. In a report from the Infection Prevention and Control Committee of Dr. Soetomo General Academic Hospital, Surabaya, there were 12 needle stick injuries (NSIs) in 2017 and 74 NSIs in 2018. Meanwhile, according to a report from the Emergency Department of Dr. Soetomo General Academic Hospital, Surabaya, there were one work-related accident in 2017 and another one in 2018.

The Emergency Department of Dr. Soetomo General Academic Hospital, Surabaya, is the most vital service unit in helping to save the lives of patients who experience medical emergencies right after entering the hospital. Because emergency management must obtain a fast response time and appropriate action, nurses in this section are often exposed to various sources of danger that can threaten life and at greater risk of accidents.

This study was conducted with the aim of analyzing safety communications and safety promotion policies that can influence the safety performance among nurses in the Emergency Department of Dr. Soetomo General Academic Hospital, Surabaya.

MATERIALS AND METHODS

This research used quantitative approach. This research was an observational cross-sectional study conducted in

the Emergency Department of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, in 2019. The population in this study were all the nurses in the Emergency Department. The inclusion and exclusion criteria were set to obtain the appropriate population size for the study. The inclusion criteria in this study were the Emergency Department nurses at Dr. Soetomo General Academic Hospital, Surabaya, who had direct contact with the patients or their environment and were willing to participate in the study.

The exclusion criteria were nurses who took time off. Based on the inclusion and exclusion criteria, the total population obtained were 208 people. The sampling technique was a simple random sampling. The sample in this study were nurses in the Emergency Department of Dr. Soetomo General Academic Hospital, Surabaya, consisting of the 1st floor nurses (Emergency Assistance), 2nd floor nurses (Obstetrics and Neonates), 3rd floor nurses (Intensive Observation), and 5th floor nurses (Operating Room). On the 4th floor there were no nurse respondents because it is the Education and Research Administration Room. The total sample in the study were 68 people.

Data supporting the results of the study were obtained through in-depth interviews with informants who knew the practice of safety management and the application of standard precautions as part of the safety behavior of nurses in hospitals. The informants in the study consisted of the Chair of the Occupational Health and Safety Committee, the Chair of the Infection Prevention and Control Committee, and the Head of the Emergency Department Nursing Room. The data collection instrument in this study was a questionnaire sheet. Observations were completed with the help of a checklist sheet and a camera for documentation, an interview guide with the help of a voice recorder, and a study of documentation.

Descriptive analysis of the study results was performed using frequency distribution table to produce a distribution and percentage of each research variable. Bivariable analysis test was performed to determine the relation of the research variables. Chi-square test was used in the bivariable test with the help of SPSS version 21, while the logistic regression test was used in the multivariable analysis test with the help of SPSS version 21. The values were categorized by the median cut-off point. If it was less than the median, it was categorized as “less”, while if it was more than or equal to the median, it was categorized as “good”.

This research was preceded with a preliminary study in order to determine the problem to be studied. The informed consent, research permit, and ethical clearance were prepared after the problem was formulated. The research had been declared ethically compliant through the certificate number 1184/KEPK/V/2019 obtained from the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital. The next steps were collecting data on personal informations through questionnaires. Data on the Big-Five personality traits were obtained through questionnaires, safety management practice through questionnaires and interviews, and safety performance data were obtained also through questionnaires and observations. The supporting data were obtained from secondary data. Then, data processing and analysis were carried out, and conclusions and suggestions were drawn.

RESULTS

Variable Identifications

Variables of the safety management practices in this study consisted of safety communications and safety promotion policies. The frequency distribution of the research variables was obtained based on the results of primary data collection from 68 respondents who were nurses in the Emergency Department of Dr. Soetomo General Academic Hospital, Surabaya. The frequency distribution of safety communications and safety promotion policies variables is shown in Table 1.

Table 1. Frequency distribution of safety communications and safety promotion policies among nurses in the Emergency Department at Dr. Soetomo General Academic Hospital, Surabaya, in 2019

Variable	Category	Frequency (n)	Percentage (%)
Safety Communications	Less	17	25.0
	Good	51	75.0
Safety Promotion Policies	Less	19	27.9
	Good	49	72.1

The measurement of safety performance was performed using a questionnaire that included the compliance and participation of respondents in work safety. The frequency distribution of safety performance research variables was obtained based on the results of primary data collection from 68 respondents who were nurses in the Emergency Department of Dr. Soetomo General Academic Hospital, Surabaya. The frequency distribution of the safety performance variable is shown in Table 2.

Table 2. Frequency distribution of safety performance among nurses in the Emergency Department at Dr. Soetomo General Academic Hospital, Surabaya, in 2019

Variable	Category	Frequency (n)	Percentage (%)
Safety Performance	Less	31	45.6
	Good	37	54.4

Table 3. The relationship between safety communications and safety promotion policies with safety performance among nurses in the Emergency Department of Dr. Soetomo General Academic Hospital, Surabaya, in 2019

Independent Variable (*Safety Management Practices*)	Dependent Variable (*Safety Performance*)				Total		*p-value*	PR
	Less		Good		N	%		
	n	%	n	%				
Safety Communications								
Less	12	70.6	5	29.4	17	100	0.035	1.895
Good	19	37.3	32	62.7	51	100		
Safety Promotion Policies								
Less	11	57.9	8	42.1	19	100	0.319	1.148
Good	20	40.8	29	59.2	49	100		

Research Variable Analysis

At this stage of analysis, bivariable analysis with Chi-square test was used to determine the relationship Table 3 shows the results of the relationship analysis between safety communications variable and safety promotion policies variable with safety performance among nurses in the Emergency Department of Dr. Soetomo General Academic Hospital, Surabaya.

According to most of the respondents, the safety communications were good. The cross-tabulation results showed that most of the respondents who stated that the safety communications were good had good safety performance (62.7%). The results of the Chi-square statistical test showed that the safety communication variable with the safety performance had a p-value of 0.035 (<0.05) indicating a significant relationship. The prevalence ratio (PR) value was 1.895 (>1) that meant the respondents who deemed the safety communications as less significant had a lower safety performance compared to respondents who stated that the safety communication was good.

According to most of the respondents, the safety promotion policies were also good. The cross-tabulation results showed that the majority of respondents who stated that the safety promotion policies were good had good safety performance (59.2%). The results of the Chi-square statistical test showed that the safety promotion policy variable with safety performance had a p-value of 0.319 (>0.05), so there was no significant relationship.

DISCUSSIONS

Most of the nurses in the Emergency Department of Dr. Soetomo Hospital, Surabaya, deemed the safety communications as good. This means that the management has implemented open door policy and open communications on safety issues, and that the management has provided an opportunity to discuss and address safety issues in meetings. In this study, statistically there was an influence of safety communications on safety performance. This was in line with a research by Putra (2017) who examined OSH communications among workers and showed the results that communication strategies have an influence in increasing workers' awareness of the importance of OSH in work environment. The results of a literature review analysis by Maulida & Damaiyanti (2021) concluded that 8 out of 15 articles found poor communications in emergency departments, especially in the patient handover process. The eight research articles conducted in the emergency departments found that the levels of patient safety were less good than in other units, and that there were significant relationships between communications and patient safety in hospital emergency rooms. A research conducted by Chen et al. (2018) showed that safety communication has a very close association with safety performance and provides better explanation regarding safety performance in Taiwan's construction industry.

The assessment of most of the nurses in the Emergency Department of Dr. Soetomo General Academic Hospital, Surabaya, showed good safety promotion policies. This can be interpreted that safety behavior is considered a positive factor for job promotion and that safety promotion activities carried out by the management are effective in creating safety awareness. In this study, statistically there was no influence of the safety promotion policies on safety performance. This was not in line with a research by Syaff (2008) who concluded that there was a quite strong influence of rewards in shaping safe behavior. This is because the existence of a reward system becomes a motivation for workers to behave safely and carry out work happily. The results of this study were also not in line with a research conducted by Ginting et al. (2021) who showed that there was an influence of OSH promotion on the employees' use of personal protective equipment and unsafe acts. A research by Andriyadi et al. (2021) was conducted on the significance of the relationships of safety talk, implementation of training, and supervision of safe behavior among PT. X workers in the construction of Mulawarman University building,

construction of Mulawarman University building, Samarinda, in 2020. The results of multivariate analysis found no influence of safety promotion activities on the safe behavior of workers. The implementation of supervision as evaluation material and reminders in the field is an effort to increase safety behavior by motivating workers through rewards and punishments. To promote safety programs effectively, the determinants of the health, safety, and environment (HSE) culture should be well understood by the workers (Tehrani et al. 2018).

Strength and limitation

This study involved all of the nurses working in the Emergency Department of the Dr. Soetomo General Academic Hospital in Surabaya. To improve safety performance, the determinants of future health, safety, and environment at each hospital,

CONCLUSION

Safety communications have influence on safety performance, while safety promotion policies have no influence on safety performance among nurses in the Emergency Department of Dr. Soetomo General Academic Hospital, Surabaya.

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Conflict of interest

The authors state that there was no conflict of interest in this study.

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Author contribution

Conceptualization: RB, NW, NS; data curation: RB, NS; methodology: RB, NW, NS; supervision: NW, ED; validation: NW, NS, ED; original draft writing: all authors; review and editing: all authors.

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Original Research Report

PHYSICAL WORKLOAD AND WORK FATIGUE AMONG TANK CAR DRIVERS AT SURABAYA INTEGRATED TERMINAL

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ABSTRACT

Every year, there are nearly a thousand times more of non-fatal accidents than fatal accidents. In Indonesia, Java Island has the highest number of traffic accidents. Work accidents have three main factors, i.e. worker factors, labor factors, and environmental factors. Fatigue is one of the conditions called silent killer. The aim of this research was to analyze the correlation of physical workload with work fatigue in tank car drivers at Surabaya Integrated Terminal. This research was an observational cross-sectional study. The sample were 205 tank car drivers fufilling the inclusion criteria as fuel tank car drivers and willing to participate in this research. The independent variable in the study was physical workload, while the dependent variable was subjective work fatigue. Data were collected using the IFRC questionnaire. The Spearman's correlation test revealed the value of Sig. (2-tailed)=0.437, indicating no significant correlation between physical workload and subjective work fatigue. The results showed that the physical workload had no correlation with work fatigue. Fatigue found among the drivers are probably induced by other factors not analyzed in this study. Companies and tank car drivers are advised to carry out work fatigue management by, for example, drinking water regularly, carrying out light exercise, and fulfilling nutritional intake.

Keywords: Physical workload; work fatigue; work accidents; traffic accidents; public health

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Highlights:

1. Physical workload had no correlation with work fatigue among tank car drivers.
2. Companies and tank car drivers are advised to carry out work fatigue management by exercising lightly and fulfilling nutritional and water intake.

INTRODUCTION

The International Labor Organization (ILO) estimates that 2.78 million workers die each year as a result of occupational accidents and diseases. About 2.4 million (86.3%) of these deaths were caused by occupational diseases, and more than 380,000 (13.7%) were caused by work accidents. Every year, there are nearly a thousand times more of non-fatal accidents than fatal accidents. An estimated number of 374 million workers are affected by non-fatal accidents each year, many of which have serious consequences for workers' earning capacity (Hämäläinen et al. 2017).

Based on the investigation of the National Transportation Safety Committee (KNKT) in 2007-2016, the highest numbers of road traffic accidents involving motorized vehicles happen in Java. West Java Province has the highest accident rate in Java with 22 accident cases. The reason is the geographical conditions, which mostly consist of hilly and mountainous areas with geometric ascents, descents, and bends. East Java Province has the second highest traffic accident rate with 13 accident cases. Central Java Province has the third highest traffic accident rate with 6 accident cases. Meanwhile, North Sumatra Province has the highest traffic accident rate in Sumatra with 5 accident cases (Saputra 2018).

The ILO states that work accidents have three main factors, i.e. worker factors (including age, education level, and work experience), labor factors (including shift work, type or work unit), and environmental factors (including physical, chemical, and biological factors) (Djatmiko 2016). The results of previous studies showed that there was a correlation between fatigue and work accidents (Tanriono et al. 2019). The results of a research by Zhang et al. (2016) showed a significant correlation between fatigue and accidents in bus drivers, and that fatigue is one of the conditions known as the silent killer.

The Surabaya Integrated Terminal, included in the work area of PT Pertamina Patra Niaga in the Jatimbalinus Region, is responsible in distributing fuel oil to all business areas in East Java, so it has a risk of tank car traffic accidents. Based on data obtained from the Surabaya Integrated Terminal, accidents occurred until November 2021 included 14 tanker truck accidents, 9 of which were active accidents. On the other hand, the number of tanker truck accidents in the Pertamina Patraniaga area throughout East Java is 27 accidents.

Based on this background, the purpose of this study was to analyze the correlation between physical workload and work fatigue in tank car drivers at the Surabaya Integrated Terminal.

MATERIALS AND METHODS

This research was an observational descriptive cross-sectional study. The aim of this study was to provide an overview of the state of the object and analyze the problems studied. This research was conducted on tanker car drivers at the Surabaya Integrated Terminal. The population in this study were 420 workers with inclusion criteria as fuel tank car drivers who were willing to participate in this research. The sample in this study were 205 people with the determination of the sample size using the Slovin formula with a degree of error of 5%. Sampling was conducted using simple random sampling technique. The use of this method provides an equal opportunity in a population to be taken as sample.

The independent variable was physical workload, while the dependent variable was work fatigue. Based on sources, data in this study were primary data and secondary data. Primary data was obtained through interviews and filling out questionnaires, while secondary data was obtained through books, articles, or previous researches with appropriate or related topics.

Data on the physical workload variable were obtained from interviews about activities carried out in a day and then the physical workload was calculated by considering the calorie needs. Based on the number of calorie needs, the physical workloads were categorized into mild (<200 kcal/hour), moderate (200-350 kcal/hour), and strenuous (>350 kcal/hour) physical workloads (Indonesian Minister of Manpower and Transmigration 2011). Data on work fatigue variable were obtained through direct questionnaires filled out by the workers using the Industrial Fatigue Research Committee (IFRC) questionnaires.

The IFRC questionnaires were assessed using a Likert scale. After being filled out by the respondents, the questionnaires were assessed by adding up the total score. The total score was classified into low (0-21), medium (22-44), high (46-67), and very high (68-90) subjective work fatigue.

The data were analyzed using univariate and bivariate analyses. Univariate analysis was carried out by presenting data in tabular form and data percentages. Meanwhile, bivariate analysis was used to see whether there was a correlation between variables using the Spearman test.

RESULTS

Based on Table 1, it can be known that the majority of respondents were >35 years old with 139 people (67.8%) and have a working period of >5 years with 123 people (60%).

Table 1. Characteristics of respondents (n=205)

Characteristics of Respondents	Total	Percentage
Age		
≤35 years old	66	32.2
>35 years old	139	67.8
Total	205	100
Working period		
≤5 years	82	40
>5 years	123	60
Total	205	100

Work fatigue can be caused by many factors, one of which is physical workload. The distribution of the workers based on the physical workload and work fatigue was as follows:

Table 2. Distribution of workers based on the physical workload

Variable	Total	Percentage
Physical Workload		
Mild	142	69.3
Moderate	63	30.7
Strenuous	0	0
Total	205	100

Based on Table 2, it can be known that the majority of respondents had mild physical workload with 142 people (69.3%). Other respondents had moderate physical workload with 63 people (30.7%).

Table 3. Distribution of workers based on the work fatigue

Variable	Total	Percentage
Work Fatigue		
Low	191	93.2
Medium	14	6.8
High	0	0
Very High	0	0
Total	205	100

Based on Table 3, it can be known that the majority of respondents experienced low subjective work fatigue with 191 people (93.2%.) Other respondents had medium work fatigue with 14 people (6.8%).

Table 4. The Correlation of Physical Workload with Work Fatigue

Variable	Work Fatigue				Total		Spear-man Rank
	Low		Medium				
	n	%	n	%	n	%	
Physical Workload							
Mild	131	92.2	11	7.8	142	100	0.437
Moderate	60	95.2	3	4.8	63	100	

Based on Table 4, it can be known that out of the tank car drivers with a mild physical workload at the Surabaya Integrated Terminal, 131 people (92.2%) had low work fatigue. Meanwhile, 11 people (7.8%) of the total respondents with mild physical workload had medium work fatigue. Out of the tank car drivers with a moderate physical workload, 60 people (95.2%) had low work fatigue. Meanwhile, 3 people (4.8%) of the total respondents with moderate physical workload had medium work fatigue.

Based on the results of the Spearman correlation test analysis where the value of α=0.05 (5%), it can be known that the value of Sig. (2-tailed)=0.437. Sig value (2-tailed)=0.437 > α=0.05. Therefore, it can be concluded that there was no significant correlation between physical workload and subjective work fatigue.

DISCUSSION

It is known that work fatigue in tanker car drivers is divided into low and moderate category. Low and moderate work fatigues that occur in tank car drivers are the results of excessive working hours. According to the Act of the Republic of Indonesia Number 13 Year 2003 concerning manpower, regulated working hours are seven hours. However, the tank car drivers' working hours are twelve hours and can even reach one full day due to the distance of fuel distribution from the Surabaya Integrated Terminal to the destinations, or due to increased orders for fuel oil. Long working hours can affect optimal work productivity and cause work fatigue (Irwanto 2020). It may increase the risk of cardiovascular disease (Kim et al. 2018).

The biggest cause of traffic accidents is human factors at 79.91% (Utomo 2019). One of the human factors is fatigue or work fatigue. Fatigue can be identified by several signs and symptoms including yawning, rubbing the eyes, dizziness, drowsiness, decreased concentration, and boredom (Caldwell et al. 2019). Fatigue can be caused by sleep factors (circadian rhythm), work factors (long working hours), and health factors (Davidović et al. 2018). Work schedule can affect work fatigue, and then work fatigue can also affect work performance (Al-Mekhlafi et al. 2021). Fatigue can lead to a decrease in productivity and affect the health of workers (Prastuti & Martiana 2017). Work fatigue can also cause work accidents due to decreased body functions while working (Fitriani et al. 2021).

Work fatigue can be overcome with work fatigue management which will have an impact on the prevalence of work accidents (Sieber et al. 2022). Work fatigue management programs must be carried out by companies and workers. Work fatigue management program for the tanker drivers includes knowing the signs of fatigue, light stretching, meeting daily drinking water needs, and optimizing rest time. Another way is taking time to be in nature which can reduce fatigue levels (Longman et al. 2021). Work fatigue management program provided by the company includes warming-up before starting work, providing drinking water and additional food, joint stretching program, providing a camera to record the driver, and providing rest areas. The company's intervention in providing additional food can increase the nutritional needs of workers so that they can work optimally, while the company's intervention with light exercise can make the workers' bodies healthier and reduce their sedentary lifestyle (Rachmah et al. 2022). The company's intervention in providing a camera for the drivers can record changes in facial expression, voice,

drivers can record changes in facial expression, voice, and posture (Murad et al. 2022). There are other ways to detect fatigue, i.e. recognizing biological changes through electroencephalogram (EEG) and electrocardiogram (ECG). These methods can describe the drivers' condition with high accuracy, but these methods require a tool that is attached to a certain body part, so it could interfere with the movement while working (Fan et al. 2022).

This study divided the physical workload experienced by the tank car drivers into mild and moderate categories. The majority of the tank car drivers had mild physical workload because they tended to not use a lot of energy and did not require great physical strength to carry out their activities. Although tank car drivers do not require much physical strength at work, tanker drivers may experience fatigue or back and neck pain due to the monotonous position of the drivers' body and awkward posture when driving (Sekkay et al. 2021). The monotonous position and awkward posture while driving can increase the drivers' fatigue, which can have an impact on absenteeism due to illness (Bláfoss et al. 2019).

The results of the study indicated that most of the physical workload of the tank car drivers was in the mild category. The physical workload categorization based on oxygen demand through calorie needs assessment cannot explain the actual exposure of workers. Other than the difficulty of the work itself, other factors that can affect calorie needs include work environment, work style, attitude, and workplace (Tarwaka 2019).

Based on the results of the study, it was found that there was no correlation between physical workload and work fatigue. The heavier the physical workload, the higher the subjective fatigue level. As in these results, light physical workloads tended to cause low work fatigue. The results of this study are in line with a research by Saputra & Hartono (2021) that there is no significant correlation between physical workload and the incidence of work fatigue in public transport drivers in Depok City, Indonesia. A research by Maulana (et al. 2021) showed similar result that there is no significant correlation between workload and the incidence of work fatigue among intercity and interprovincial (AKAP) bus drivers in PT Eka Sari Lorena Transport Tbk, Bogor, Indonesia.

Fatigue can be caused by factors that may be work-related, non-work-related, or a combination of both, and can accumulate over time. When the body is inadequate to recover from fatigue, the fatigue can continue to accumulate (Gabriel et al. 2018).Work fatigue is generally caused by external factors of workload, work itself, or organization (working hours, breaks, shifts, night work, etc.), work environment (physical, chemical, biological, ergonomic, and psychological), physical factors (age, gender, height, condition, nutritional status), and psychological factors (motivation, job satisfaction, desire, etc.) (Russeng in Mahardika 2017). The results showed that the physical workload variable was not related to work fatigue due to the fact that there were probably different variables that induced the drivers' fatigue to be minimum, or different variables that now have been no longer analyzed, so they could not outline the causes of the fatigue.

Strength and limitation

Therefore, the limitation of this study is the lack of other variables to study because it only examines one factor while, there are many factors related to work fatigue. It is recommended for future research to examine other factors that have not been studied such as organizational factors, physical factors, and psychological factors.

CONCLUSION

The results of this study showed that there is no correlation between the physical workload variable work fatigue. It is due to the fact that there are probably different variables that induced the drivers' fatigue to be minimum. Another possibility of it is that there are different variables that now have been no longer analyzed. Therefore, the causes of the fatigue cannot be outlined. Variables that may have an effect but had not been researched in this study were individual characteristics, working hours, and work motivation. Companies and tank car drivers are advised to carry out work fatigue management by meeting the needs of drinking water, exercising lightly, and fulfilling nutritional intake.

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Conflict of interest

Tasya Endah Milinia and Dani Nasirul Haqi declare no conflicts of interest. Siti Arum Alia is an employee in PT Pertamina Patra Niaga Regional Jatimbalinus.

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Author Contribution

TEM wrote the manuscript with a support from DNH. SAA conceived the original idea.

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Original Research Report

HEALING OF RADIATION DERMATITIS WITH OZONATED *Aloe vera* OIL BY INCREASING PDGF AND EPIDERMAL THICKNESS IN SPRAGUE-DAWLEY RATS

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ABSTRACT

The long-term use of corticosteroids as a standard treatment for skin disorders, such as radiation dermatitis, can cause many side effects. Alternatively, ozonated Aloe vera *oil may replace corticosteroids due to its fewer side effects and benefits in wound healing process. Re-epithelialization and the formation of growth factors, such as platelet-derived growth factor (PDGF), play an important role in the healing of dermatitis wound. This study intended to demonstrate the effect of ozonated* Aloe vera *oil to improve the healing of radiation dermatitis wound by increasing PDGF expression and epidermal thickness. This study used a post-test only control group design. A sample of 36 Sprague-Dawley rats was divided into 6 groups (C1=without treatment, C2=2.5% hydrocortisone ointment, P1=pure* Aloe vera *(AV), P2=300 mg/mL ozonated* Aloe vera *(OAV), P3=600 mg/mL OAV, P4=1200 mg/mL OAV. The expression of PDGF was assessed using Allred scoring with immunohistochemical staining, whereas the epidermal thickness was assessed using hematoxylin and eosin (H&E) staining at 400x microscopic magnification. The PDGF expression and epidermal thickness between the control and the treatment groups showed significant differences using a Kruskal-Wallis test (P=0.001) and one-way ANOVA test (P<0.001). The groups that was given ozonated* Aloe vera *oil had higher average of PDGF expression and thicker epidermis than the other groups. The Spearman's correlation test showed a strong positive relationship (p<0.001 and r=0.709) between the two variables. In conclusion, ozonated* Aloe vera *oil improves healing of radiation dermatitis wound by increasing PDGF expression and epidermal thickness.*

Keywords: *Ozonated* Aloe vera*; radiation dermatitis; platelet dervied growth factor (PDGF); epidermal thickness; biomedics; health risk ; increasing life expectancy*

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Highlights:

1. This study demonstrated the effect of ozonated Aloe vera oil in the healing of radiation dermatitis wound.
2. Groups that received no treatment was compared with groups that received treatment using 2.5% hydrocortisone ointment and ozonated Aloe vera.
3. Ozonated Aloe vera oil improves the healing of radiation dermatitis wound by increasing PDGF expression and epidermal thickness.

INTRODUCTION

Dermatitis is one of the most common skin diseases and has various causes, one of which is radiation exposure (Paramata & Abas 2019, Prakoeswa et al.2021, Ginting et al. 2021).). The side effects of ionizing radiation include various disorders, such as skin fibrosis, skin cell aging, skin cell necrosis, and the initiation of epidermal and dermal inflammatory responses (Singh et al. 2016, Robijns & Laubach 2018, Rosenthal et al. 2019). Radiation causes complications,

such as delayed wound healing, reduced aesthetic appeal, and decreased quality of life. It was known that ~95% of cancer patients undergoing a radiotherapy have radiation dermatitis (Spałek 2016, Wang et al. 2020). Radiation dermatitis or radiodermatitis is the result of an imbalance of pro-inflammatory and profibrotic cytokines. It decreases the number of growth factors, such as platelet-derived growth factor (PDGF), epidermal growth factor (EFG), transforming growth factor-β (TGF-β), and fibroblast growth factor (FGF) (Han et al. 2020, Vadarli & Angelo-Khattar 2020).

Topical corticosteroids are standard drugs commonly used to treat various skin diseases, one of which is radiation dermatitis (Gabros et al. 2022). However, the use of topical corticosteroids in the long term was found to have side effects both locally and systemically. In this era, many alternative drugs have been developed to be used as substitutes with minimal side effects to corticosteroids (Coondoo et al. 2014, Kumar et al. 2019). *Aloe vera* is one of the alternatives for treating various skin diseases (Rao et al. 2017). Its healing properties are related to the compounds it contains, the glucomannan, which can influence fibroblast growth factors, increase cell activity and proliferation, and stimulate collagen production (Hashemi et al. 2015, Vahlepi et al. 2020, Tamba et al. 2020).

Ozone (O3) is a classic and sterilizing oxidant that is widely applied in clinical practices. Ozone has antimicrobial, antioxidant, immunoregulatory, epi-genetic modification, biosynthetic, analgesic, and vasodilatation effects. Ozone therapy in topical preparations can be used as an addition or alternative to the treatment of various types of skin wounds (Anzolin et al. 2020, Zeng et al. 2020).

This study differed from previous studies that used gentamicin ointment as control and ozonated *Aloe vera* oil of 600, 1200, and 1800 mg/mL doses on full thickness wounds (Vahlepi et al. 2020). In this study, ozonated *Aloe vera* was used at doses of 300, 600, and 1200 mg/mL as the treatment groups and 2.5% hydrocortisone ointment as the positive control. The effect of using ozonated *Aloe vera* oil as treatment for radiodermatitis has not been widely studied, while the long-term use of topical corticosteroid has many side effects. Therefore, this study was conducted to investigate the effect of ozonated *Aloe vera* oil in radiation dermatitis wound healing observed from the PDGF expression and epidermal thickness.

MATERIALS AND METHODS

This research was an experimental study using a post-test only control group design from August to September 2021. The use of experimental animals in this study complied to the animal ethics approval No. 66/EC/H/FK-UNDIP/VII/2021 obtained from the Health Research Ethics Commission, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia. All Sprague-Dawley rats used as the experimental animals were treated and cared for according to animal husbandry standards. External radiation was administered to all experimental animal groups. Radiodermatitis treatment was differentiated by treatment groups.

The experimental animal research subjects were 36 Sprague-Dawley rats divided randomly into 6 groups (6 rats each) consisting of two control groups and four treatment groups. The inclusion criteria for the experimental animals were male rats, aged 2-3 months and weighed 150±50 grams, obtained from the Experimental Animal Laboratory, Universitas Sultan Agung, Semarang. The rats were habituated in individual cages for a week with a 12-hour period of light (06.00 to 18.00) and a 12-hour period of dark (18.00 to 06.00) at a room temperature of 28.0±2.0 ^{o}C. Food and water for the rats were given ad libitum. External radiation treatment to create radiation dermatitis wounds on the rats was carried out after acclimatization. In this study, after radiation, three rats died from dehydration.

Radiation dermatitis was created by irradiating a 4x4 cm area on the rats' back with gamma rays. The radiation dose used was 7 Gy in a single dose, which in the calculation of the equipment used is equivalent to 5 minutes. Radiation was administered using a Siemens Primus linear accelerator radiotherapy machine (Concord, CA, USA) at Ken Saras Hospital, Semarang, Indonesia. Prior to the irradiation, the rats were anesthetized with a dose of 80 mg/kgBW Ketamine (Ket-A-100, Peru) and 10 mg/kgBW Xylazine (Xyla, Indonesia) via the intraperitoneal route. After the irradiation, the rats were kept in a clean and warm place with the help of a lamp until they recovered from the effects of anesthetics.

The Common Terminology Criteria for Adverse Events (CTCAE version 5.0) from the National Cancer Institution categorizes dermatitis levels into five levels (National Institutes of Health-National Cancer Institute 2017). The severity of radiation dermatitis is very important to note in determining the appropriate management.

Table 1. Dermatitis levels according to the Common Terminology Criteria for Adverse Events (CTCAE version 5.0).

Level 1	Faint erythema or dry desquamation
Level 2	Moderate to sharp erythema; uneven moist desquamation, mostly confined to skin folds and folds; moderate edema
Level 3	Moist desquamation in areas other than skin folds and folds; bleeding caused by minor trauma or abrasion
Level 4	Life-threatening consequences; full thickness skin necrosis and ulceration of the dermis; spontaneous bleeding from the involved site; skin graft indicated
Level 5	Death

The ozonated *Aloe vera* oil was manufactured at the Plasma Research Laboratory of Universitas Diponegoro. The tools used to produce the ozonated oil were an ozone generator and a magnetic stirrer. The oil was obtained by turning the ozone produced by ozone generator into oil. Potassium iodide titration method was used to measure the ozone levels in the ozonated oil. In this study, ozonated *Aloe vera* was used at 300, 600, and 1200 mg/mL doses.

In this study, the radiation dermatitis had been formed by comparing normal skin tissue and the irradiated skin, where there was a change in skin color, desquamation, amount of PDGF expression, TGF beta, epidermal thickness, and others on the irradiated skin. In this study, dermatitis level 1 occurred in the experimental animals. Ran et al. (2004) stated through their study that gamma ray irradiation of 1-8 Gy can cause radiation wound already in rats.

Ozonated *Aloe vera* oil was applied to the radiation dermatitis wounds twice a day, with 1,045 mL thinly covering the entire wound surface. A previous study applied a variety of an ozonated oil with a dose of 8 microliters on round wounds with a diameter of 3.5 mm (Valacchi et al. 2011). In this study, the diameter of the wound was 40 mm, so the required doses were 313.478 mg/mL, 626.956 mg/mL, and 1253.91 mg/mL which were equivalent to 1.045 mL per application. The interventions were carried out for seven days until the experimental animals were subjected to microscopic and immunohistochemical assessments. The inter-ventions were given based on the division of treatment groups, i.e. C1=no therapy/intervention (negative control) and C2=2.5% hydrocortisone ointment (positive control), P1=*Aloe vera* oil, P2=300 mg/mL ozonated *Aloe vera* (OAV), P3=600 mg/mL OAV, and P4=1200 mg/mL OAV.

Excision was carried out on day 7 for each treatment group. After the Sprague-Dawley rats were terminated by placing them in a closed room and then giving them chloroform inhalation, wound tissue excision process can be carried out. The excision was performed on the widest part of the wound tissue along with the surrounding healthy skin tissue. The process of cutting the tissue was using a microtome by placing it on a paper and fixing it in 10% buffered formalin. It was followed by the immunohistochemical staining and hematoxylin-eosin staining were carried out.

Wound tissue sections (slices) were placed in neutral buffered formalin solution and fixed for 18-24 hours. The tissue fixing agent was removed with distilled water for an hour. The tissue sections were immersed in graded alcohol concentrations (80%, 95%, 95%, absolute alcohol I, II, III) for an hour each and then cleaned.

Tissue preparations were put into paraffin for two hours in an incubator at 56-58ºC. Tissues in the paraffin were sectioned to a thickness of 3-6 μm using a microtome and then floated in water at 40ºC. Tissue sections were transferred to a microscopic slide and dried overnight at room temperature until they were ready for hematoxylin-eosin staining and PDGF immunohistochemical staining.

PDGF expression assessment

PDGF immunohistochemical staining was performed at the Laboratory of Anatomical Pathology, Universitas Sebelas Maret, Surakarta, Indonesia. PDGF expression readings were performed by an anatomical pathologist at Universitas Diponegoro. Cells in wound tissue expressing the PDGF appeared brown on the immunohistochemical staining. These cells were semi-quantitatively measured using the Allred score by examining the percentage per 100 stained cells and the staining intensity. Then, the average per 100 cells/field was calculated from five fields of view at 400x magnification.

Epidermal thickness assessment

Epidermal thickness measurement was performed by an anatomical pathologist at Universitas Diponegoro. Epidermal thickness was measured from the thickness of the epidermis—from the stratum corneum to the stratum basale in three fields of view using a Nikon microscope with 400x magnification. Then, the mean of the results were calculated. Epidermal thickness was calculated in micrometers (μm) and processed digitally using Indomicro software.

Data analysis

Data analyses, including the descriptive analysis and hypothesis testing, were carried out using the SPSS 21.0 program. In the descriptive analysis, PDGF expression and epidermal thickness were presented in the form of a table of mean and standard deviation (SD). The Shapiro-Wilk test was used to examine the data normality. The Kruskall Wallis test was used to examine the differences in PDGF expression between each group. It turned out that the data were not normally distributed, so it were continued with the post-hoc Mann Whitney test. ANOVA test was used to examine the differences of epidermal thickness between each group because the data were normally distributed. A correlation test between variables was carried out using the Spearman correlation test. The significance limit was $p \leq 0.05$ with a 95% confidence interval.

RESULTS AND DISCUSSION

PDGF expression

Table 1 presents the results of the immunohistochemical analysis of PDGF expression from the Sprague-Dawley rats' dermatitis skin sections.

Table 1. Data normality comparison of the use of hydrocortisone, pure *Aloe vera,* and ozonated *Aloe vera* oil in radiation dermatitis healing based on the Shapiro-Wilk test

Group	N	Mean ± SD	Median (min–max)	p	Note
C1	5	4.04±0.50	4.2 (3.2–4.4)	0.090	Normal
C2	5	4.52±1.06	4.8 (3.0–5.8)	0.913	Normal
P1	5	5.60±1.02	5.2 (5.0–7.4)	0.007	Abnormal
P2	6	5.73±0.39	5.9 (5.0–6.0)	0.033	Abnormal
P3	6	6.17±0.97	5.8 (5.4 8.0)	0.057	Normal
P4	6	6.40±0.70	6.3 (5.6–7.6)	0.478	Normal

Table 2. Defferences of PDGF expression among the use of hydrocortisone, pure *Aloe vera,* and ozonated *Aloe vera* oil in radiation dermatitis healing based on Kruskal Wallis test

Group	Mean ± SD	p
C1	4.04±0.50	0.001
C2	4.52±1.06	
P1	5.60±1.02	
P2	5.73±0.39	
P3	6.17±0.97	
P4	6.40±0.70	

Table 3. Significance of hydrocortisone, pure *Aloe vera,* and ozonated *Aloe vera* oil in radiation dermatitis healing based on post-hoc Mann Whitney test

Group		p
I	II	
C1	C2	0.401
	P1	0.009*
	P2	0.006*
	P3	0.006*
	P4	0.006*
C2	P1	0.113
	P2	0.026*
	P3	0.028*
	P4	0.010*
P1	P2	0.264
	P3	0.081
	P4	0.066
P2	P3	0.681
	P4	0.080
P3	P4	0.329

(*)=siginificant

These results presented that the groups receiving ozonated *Aloe vera* oil had higher PDGF expression than other groups. These results are consistent with a previous study that wound healing and angiogenesis were better in the groups receiving ozonated oil (Valacchi et al. 2011). The effect of giving ozonated *Aloe vera* oil on PDGF expression was proven to be better than 2.5% hydrocortisone ointment as a positive control. Ozone in the *Aloe vera* oil activates the collagen synthesis and fibroblast proliferation that help wound healing.

Ozone can assist tissues to be adaptive to oxidative stress, stimulate endogenous antioxidants, and protect against tissue damage. The reaction of polyunsaturated fatty acids and small amounts of water in the upper layer of the dermis will form reactive oxygen species (ROS) and lipo-oligopeptides including H_2O_2 that helps the healing process of wounds (Anzolin et al. 2020). Besides that, the release of endogenous factors, such as PDGF and TGF-β, as well as the re-epithelialization process, were influenced by fibroblast. The content in *Aloe vera* plant shows its effect on wound healing by activating macrophages. In addition, ozonated *Aloe vera* oil can induce the expression of PDGF and TGF-β of epidermal keratinocyte and dermal fibroblast in wound sites (Valacchi et al. 2011, Hashemi et al. 2015, Atik et al. 2019, Zeng et al. 2020, Vahlepi et al. 2020, Tamba et al. 2020). PDGF will transduce its signal through macrophages which will increase the thickness of the dermis and epidermis, thus helps accelerating the wound healing process (Kim et al. 2009, Hashemi et al. 2015, Heldin et al. 2018, Atik et al. 2019, Zeng et al. 2020, Vahlepi et al. 2020, Tamba et al. 2020). *Aloe vera* has fewer side effects than hydrocortisone as a chemical drug. Hydrocortisone has several side effects, including atrophic changes in the skin, infection, ocular changes, pharmacological side effects, and other non-specific side effects (Hengge 2017, Williams 2018).

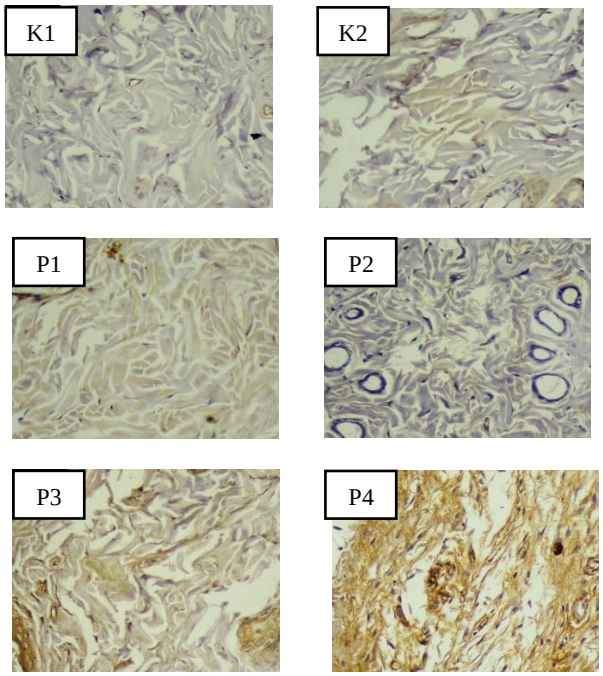

Figure 1. Results of PDGF immunohistochemical staining.

Epidermal thickness

Table 4 presents the results of histological examination using H&E staining that showed the epidermal thickness of wound tissue sections of the experimental animals.

Table 4. Epidermal thickness descriptive data and the Shapiro-Wilk normality test results

Group	N	Mean ± SD	Median (min–max)	P	Note
C1	5	12.32±1.71	12.02 (10.69–15.21)	0.164	Normal
C2	5	13.31±2.38	12.65 (10.15–16.09)	0.707	Normal
P1	5	14.83±3.43	13.60 (11.85–20.42)	0.287	Normal
P2	6	22.82±4.18	23.52 (16.17–27.46)	0.756	Normal
P3	6	25.29±7.97	23.97 (12.70–35.46)	0.573	Normal
P4	6	29.83±6.64	29.73 (20.67–40.22)	0.990	Normal

Table 5. The result of one-way ANOVA test

Group	Mean ± SD	p	Levene
C1	12.32±1.71	<0.001	0.150
C2	13.31±2.38		
P1	14.83±3.43		
P2	22.82±4.18		
P3	25.29±7.97		
P4	29.83±6.64		

Table 6. The result of post-hoc least significant difference (LSD) test

Group		p
I	II	
C1	C2	0.762
	P1	0.445
	P2	0.002*
	P3	<0.001*
	P4	<0.001*
C2	P1	0.643
	P2	0.005*
	P3	0.001*
	P4	<0.001*
P1	P2	0.016*
	P3	0.002*
	P4	<0.001*
P2	P3	0.411
	P4	0.025*
P3	P4	0.136

(*)=significant

Table 5 shows that the highest mean of epidermal thickness was found in the group that received 1200 mg/mL ozonated *Aloe vera* oil. This can be due to the role of ozone which has an “oxidative killing" effect on organisms. The greater the amount of ozone, the better it will react to the wound. P2, P3, and P4 groups receiving ozonated *Aloe vera* oil showed that the epithelium appeared thicker than the group without any therapy (negative control), 2.5% hydrocortisone group (positive control), and *Aloe vera* group. This was in accordance with a previous study that ozonated oil can stimulate re-epithelialization better than non-ozonated oil (Valacchi et al. 2011, Kumar et al. 2019, Tamba et al. 2020).

The content in *Aloe vera* can stimulate cell proliferation, increase collagen production, affect fibroblast growth factors, and increase keratinocyte multiplication and migration, so it can help the process of forming new epidermis. This content also plays a role in increasing the occurrence of DNA synthesis and stimulating the binding of epithelial growth factor receptors with ligands, which will activate signals to proliferate reepithelialization (Kumar et al. 2019, Alfiaturrohmah et al. 2020, Tamba et al. 2020). Through the process of activation and increased metabolic activity, the amount of epidermal growth factor (EGF) receptor expression will increase. Epidermal closure of the wound tissue will occur more actively, so the re-epithelialization process is faster (Hashemi et al. 2015, Alfiaturrohmah et al. 2020).

The wound healing process affected by ozone exposure in wound site can also be seen through its ability in reducing bacterial infections, improving dermal wound healing disorders, and increasing oxygen tension (Kim et al. 2009). Ozone reacts with polyunsaturated fatty acids (PUFAs) to form reactive oxygen species (ROS), such as hydrogen peroxide, by activating redox transcription factors, such as NFkB, which induce growth factor synthesis and accelerate the cell cycle (Kim et al. 2009, Valacchi et al. 2011).

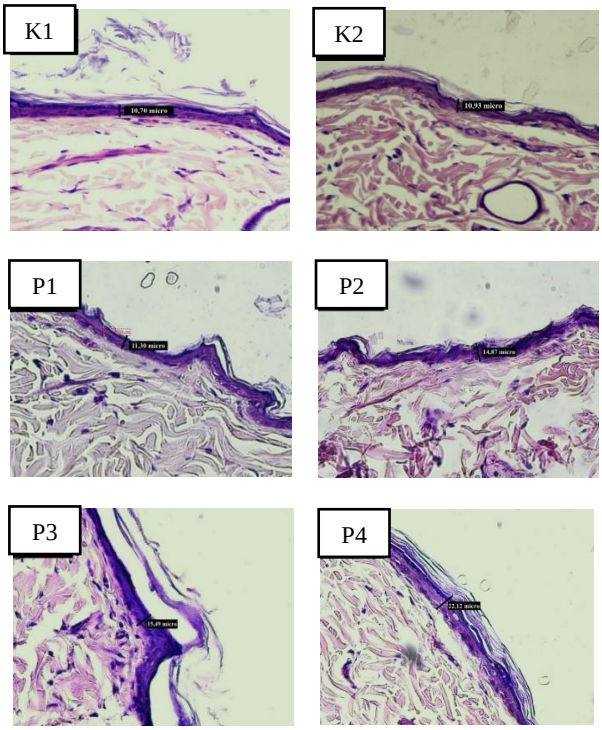

Figure 2. Epidermal thickness by H&E staining of wound tissue in the Sprague-Dawley rats

PDGF and epidermal thickness correlation test

Correlation tests were performed to determine the relationship between the variables, i.e. PDGF

expression and epidermal thickness. The results are shown in Table 7 and Table 8.

Table 7. PDGF expression and epidermal thickness descriptive data and the Shapiro-Wilk normality test result

Variable	Mean ± SD	Median (min–max)	p	Information
PDGF	5.47±1.13	5.6 (3.0–8.0)	0.623	Normal
Epidermal thickness	20.30±8.20	19.96 (10.15–40.22)	0.015	Abnormal

The Shapiro-Wilk normality test result (Table 7) showed that the PDGF expression data were normally distributed ($p>0.05$). Data obtained from the epithelial thicknes were not normally distributed ($p<0.05$), so the Spearman's Rho correlation test was performed.

Table 8. The Spearman's Rho correlation test of PDGF on epidermal thickness results

Variable	p	r	Information
PDGF			
Epidermal thickness	<0.001	0.709	Significant, positive, strong

The Spearman's Rho correlation test presented $p\leq0.001$, indicating a significant correlation between PDGF expression and epidermal thickness. The results of this study indicated the direction of strong positive correlation (Table 8).

The glycoproteins contained in *Aloe vera*, as well as the ozone content in ozonated *Aloe vera* oil, can stimulate cell proliferation and increase the expression of epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FBF), and other growth factors that will stimulate epithelial formation (Hashemi et al. 2015, Atik et al. 2019). PDGF will induce the proliferation and migration of fibroblasts and keratinocytes which can affect the wound healing process by increasing the thickness of the dermis and epidermis (Jacobson et al. 2017). This indicates that the occurrence of keratinocyte cell proliferation may increase or decrease depending on the number and type of growth factors, thus PDGF production leads to more proliferation which accelerates the re-epithelialization process (Kim et al. 2009, Valacchi et al. 2011).

Strength and limitation

The limitations of this study were the equipment used and the high cost, so the radiation beam was administered only once (single dose). Moreover, the cost of immunohistochemical examination was relatively high. This study was expected to be used as the basis for clinical trials of ozonated Aloe vera products as wound healing therapy in humans according to evidence-based medicine.

CONCLUSION

Radiation dermatitis, also known as radiodermatitis, happens as a result of skin lession due to radiation exposure. Ozonated *Aloe vera* can improve PDGF expression and epidermal thickness. Immunohisto-chemical and histological examinations using H&E staining to asses PDGF expression and epidermal thickness exhibit that topical ozonated *Aloe vera* oil can increase PDGF expression and epidermal thickness in healing of radiation dermatitis wound. The healing of radiation dermatitis wound with topical administration of ozonated *Aloe vera* oil is better than without any therapy and with topical administration of 2.5% hydrocortisone.

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Conflict of interest

The authors declare that there was no conflict of interest regarding publication of this study.

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Author contribution

WPU participated in the design of the study, manuscript preparation, and manuscript writing. YWP proposed the main idea, participated in the design of the study, methodology, and supervision. CHNP participated in the idea building and formal analysis. US contributed in the supervision, while NS contributed in the supervision and formal analysis. MR and MN served as the scientific advisors on the *Aloe vera* oil.

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Original Research Report

ANTIFUNGAL ACTIVITY OF KINAR (*Kleinhovia hospita* L.) LEAF ETHANOL EXTRACT AGAINST *Malassezia furfur*

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ABSTRACT

Pityriasis versicolor is a superficial dermatomycosis that can decrease human self-confidence. This infection is caused by the fungus Malassezia furfur. *Eighty percent of recurrence cases after treatment and resistance to antifungal agents were found. Therefore, alternative medicine is needed. Kinar (*Kleinhovia hospita *Linn.) is a tropical plant that have bioactive compounds, such as alkaloids, flavonoids, tannins, and saponins. The purpose of this study was to determine the effectiveness of kinar leaf extract against the growth of* Malassezia furfur. *This research was a laboratory experimental study using paper disc diffusion method. Kinar leaves (green and yellow leaves) were macerated using 96% ethanol and made into concentrations of 10%, 20%, 40%, 60%, 80%, and 100%. As much as 200 mg of ketoconazole was used as a positive control and distilled water as a negative control then tested on* Malassezia furfur *using Sabouraud dextrose agar (SDA). The parameter observed was the clear zone formed around the paper disc. The tests and observations showed that there was a clear zone formed around the paper disc. It means that the kinar leaf extract cannot inhibit the growth of* Malassezia furfur.

Keywords: *Antifungal;* Kleinhovia hospita *L.;* Malassezia furfur*; tropical disease; human and medicine*

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Highlights:

1. Alternative medicine is needed for pityriasis versicolor because there are recurrence cases and resistance to antifungal agents.
2. Kinar leaf ethanol extract cannot inhibit the growth of Malassezia furfur.
3. Further studies are suggested on the type of solvent and appropriate concentration to attract more bioactive compounds.

INTRODUCTION

Dermatomycosis or superficial mycosis is a disease of the skin, nails, and hair caused by dermatophyte and non dermatophyte fungi. The incidence of superficial fungal infections worldwide is common, with a progressive increase, and has affected about 20-25% of the global population. Generally, dermatomycoses occur in tropical countries. Skin fungal infections are estimated to have a high prevalence in Indonesia, such as pityriasis versicolor (PV) or commonly known as *panu* in Indonesian (World Health Organization 2005, Yusuf et al. 2017, Araya et al. 2021).

In places with high humidity and an average temperature of 28-33˚C, it is common to sweat easily and it can cause skin conditions. Other factors that can increase the risk of fungal infections are poor sanitation, lack of health knowledge, and densely populated or low socio-economic environment. Under these conditions, *Malassezia furfur* can easily infect humans, even though this fungus is actually a normal flora (Yusuf et al. 2017, Ariana 2018).

Researches conducted by public medical teaching hospitals in Indonesia often find the cases of pityriasis versicolor. In the Outpatient Unit of the Department of Dermatology and Venereology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, the number of superficial mycoses cases found was 502 (5.47%) in 2011, 312 (4.91%) in 2012, and 322 (5.90%) in 2013. The examination in 2011-2013 using 20% potassium hydroxide (KOH) and Parker's ink resulted in the most Dermatology and Venereology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, the number of superficial mycoses cases found was 502 (5.47%) in 2011, 312 (4.91%) in 2012, and 322 (5.90%) in 2013. The examination in 2011-2013 using 20% potassium hydroxide (KOH) and Parker's ink resulted in the most commonly found diagnosis which was pityriasis

versicolor in 284 patients (Rosida & Ervianti 2017). In the period 2013-2015, the Outpatient Unit of the Pediatric Dermatology, Department of Dermatology and Venereology, Dr. Soetomo Hospital treated 43,073 patients, 94 (28.3%) of which were diagnosed with pityriasis versicolor and 1 (0.3%) with tinea pedis (Sheiladji & Zulkarnain 2016). The Outpatient Clinic of Dermatology and Venereology of Prof. Dr. R.D. Kandou Central General Hospital, Manado, Indonesia, found 36 (0.87%) pityriasis versicolor diagnosed cases in January-December (Isa et al. 2016).

Previous research conducted by Gupta & Foley (2015) revealed that pityriasis versicolor is still difficult to cure because 80% of relapses occur after treatment within two years. The infections caused by *Malassezia furfur* can be treated with antifungals given via systemic or topical routes. The first treatment for pityriasis versicolor is the topical antifungals. However, *Malassezia furfur* strains were found to be resistant to antifungals, such as the azole group. Kalyani et al. (2014) conducted a study on 100 samples of pityriasis versicolor patients, with 41 tested positive for KOH and 32 positive for culture. Of these 32 patients, 23 (69%) were found to have recurrent infections. From the isolates of *Malassezia furfur* in the study, 75% isolates were found to be sensitive to ketoconazole and 100% isolates were susceptible to fluconazole and clotrimazole. Circulars issued by the National Agency of Drug and Food Control of the Republic of Indonesia (*Badan Pengawas Obat dan Makanan*/BPOM RI) and the United States Food and Drug Administration (U.S. FDA) revealed that ketoconazole causes hepatotoxicity (Kang et al. 2019). In addition, pityriasis versicolor has a bad stigma in society and can affect the quality of life and self-confidence, so most patients who come for treatment complain about their appearance (Gupta et al. 2002, Radila 2022). Currently, there is no treatment to provide satisfactory results in treating the signs and symptoms of pityriasis versicolor and especially its recurrence (Mahmoud et al. 2014). It is necessary to look for the latest alternative treatment with antifungal activity that is certainly better and has very little toxicity to prevent the recurrence of pityriasis versicolor. The treatment mentioned is the use of tropical plants as herbal medicines because until now there are still many people using plants as alternative medicines (Sears & Schwartz 2017).

Tropical plants can produce natural chemical compounds, such as pesticides, insecticides, antifungals, and cytotoxics (Valli et al. 2012, Vleminckx et al. 2018). Plant secondary metabolites also play an important role in determining the biological activity in the use of medicinal plants. Therefore, identification and isolation of secondary metabolites is important for standardization and improvement of plant quality.

Kinar plants (*Kleinhovia hospita* Linn.) have been widely used by Indonesian people as medicine, starting from the trunk, bark, until the leaves. A study has isolated more than 24,000 secondary metabolic structures of *Kleinhovia hospita* Linn. and evaluated their biological activities (Gaffar & Mamahit 2010). All parts of this tree produce natural chemical compounds, such as cyanogens, alkaloids, proanthocyanins, cyanidins, flavanols, kaempferols and quercetins and saponins (Hasanuddin & Andini 2017). In Negeri Latu, Amalatu District, West Seram Regency, Maluku, Indonesia, yellow leaves of this plant are commonly used by the local people as a topical medicine mixed with whiting pounded in a coconut shell.

MATERIALS AND METHODS

Pure culture of *Malassezia furfur* was obtained from Indi Laboratorium, then tested and identified in the Microbiology Laboratory of Faculty of Agriculture, Universitas Mulawarman, Indonesia. The culture was grown on Sabouraud dextrose agar (SDA) supplemented with 1% (v/v) pure olive oil, followed by an incubation at 37˚C for 2-7 days. The *Malassezia* strains were maintained on the same medium.

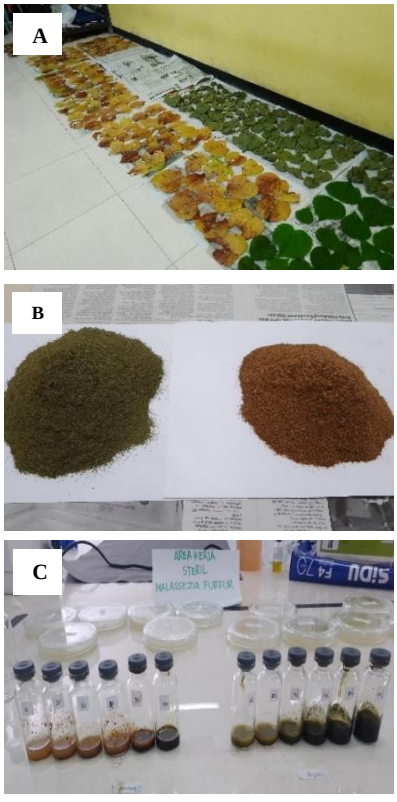

Figure 1. Sample and extract preparation: (A) the drying stage of green and yellow kinar leaves; (B) green and yellow kinar leaves in powder form; (C) green and yellow kinar leaf ethanol extracts in various concentrations

The culture was examined under the microscope using 10% KOH and methylene blue, and then the characters were recorded (Figure 1). Two types of kinar leaves (green leaves and yellow leaves) collected were washed, shade dried, and ground into a fine powder. The powder was weighed into 200 g portions, then each of them was soaked in 1,100 mL 96% ethanol for a day with intermittent stirring. The extracts were filtered and concentrated using rotary evaporator. The concentrated extracts were subjected to determination of weight per milliliter. The extracts were made into six concentrations, i.e. 10%, 20%, 40%, 60%, 80%, and 100%.

The culture of *Malassezia furfur* was swabbed over the Sabouraud dextrose agar using sterile cotton buds. Blank paper discs dipped in various concentration of kinar leaf extracts were placed equidistantly around the margin of the plates. The positive and negative control were 200 mg ketoconazole and maintained with filter paper discs dipped in distilled water. Two replicates were maintained. The plates were incubated at 37 °C and the inhibition zone was observed after 7 days.

RESULTS

This study used samples of green and yellow kinar leaves that were extracted with maceration method using 96% ethanol solvent and divided into several concentrations of 10%, 20%, 40%, 60%, 80%, and 100%. The ethanol extract of kinar leaves was then tested for the inhibitory activity against *Malassezia furfur* by disc diffusion method.

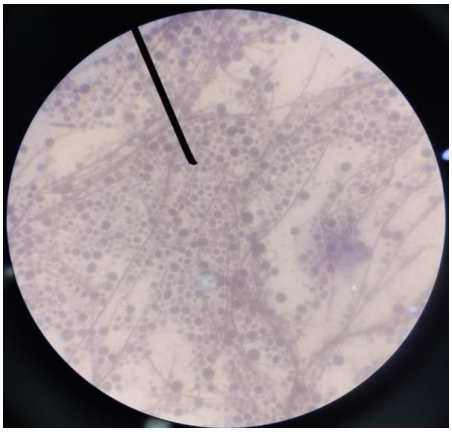

Figure 2. Morphological character of *M. furfur* (40x magnification). The presence of hyphae and spores generally exhibits the characteristic appearance of "spaghetti and meatballs"

Table 1 shows the results of the inhibition zone measurement on the green and yellow kinar leaf extracts against the growth of *Malassezia furfur*. The green and yellow kinar leaf extracts in all concentrations showed that the diameter of the inhibition zone was 0 and no clear zone was formed (Figure 3), indicating that the kinar leaf ethanol extracts were not able to inhibit the growth of *Malassezia furfur*.

Pathogenic fungi	Concen-tration %	Green leaf extracts		Yellow leaf extracts	
		Zone of inhibiton (mm)	Zone of inhibition category	Zone of inhibition (mm)	Zone of inhibition category
Malassezia furfur	10%	0	No inhibition zone	0	No inhibition zone
	20%	0	No inhibition zone	0	No inhibition zone
	40%	0	No inhibition zone	0	No inhibition zone
	60%	0	No inhibition zone	0	No inhibition zone
	80%	0	No inhibition zone	0	No inhibition zone
	100%	0	No inhibition zone	0	No inhibition zone
	+	0	No inhibition zone	0	No inhibition zone
	-	0	No inhibition zone	0	No inhibition zone

Table 1. Antifungal activities of *Kleinhovia hospita* L. leaf extracts against *M. furfur*

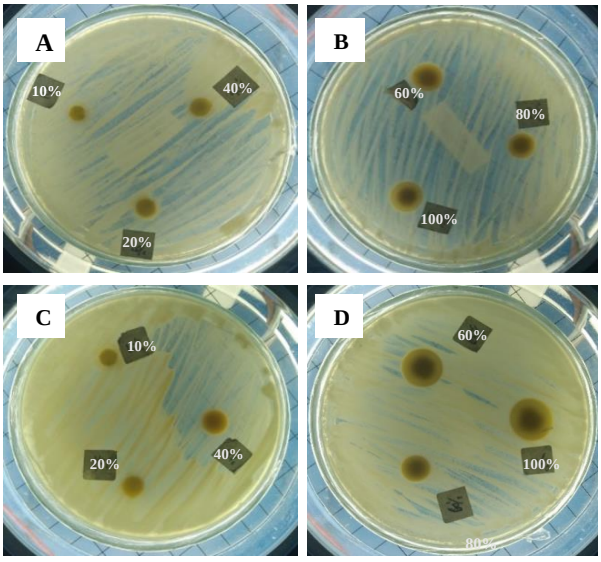

Figure 3. Antifungal assay: (A & B) inhibition zone of green kinar leaf extracts against *M. furfur*; (C & D) inhibition zone of yellow kinar leaf extract against *M. furfur*

Table 1 shows the results of the inhibition zone measurement on the green and yellow kinar leaf extracts against the growth of *Malassezia furfur*. The green and yellow kinar leaf extracts in all concentrations showed that the diameter of the inhibition zone was 0 and no clear zone was formed

DISCUSSION

Malassezia plays a pathogenic role in various skin diseases like pityriasis versicolor, seborrheic dermatitis, folliculitis, atopic dermatitis, and dandruff (Prohic et al. 2016). Generally, the treatment of these diseases consisted of azole drugs, such as fluconazole and ketoconazole. However, the increasing use of antifungal agents has led to adverse effects including severe toxicity in mammalian cells and urticaria (Kyriakidis et al. 2016). *Malassezia furfur* related diseases are often refractory to therapy and require extensive use of antifungal and anti-inflammatory drugs that can cause drug resistance. Therefore, it is important to find safe and effective treatment without side effects (Kyriakidis et al. 2016, Sivasankar et al. 2017, Kulkarni et al. 2020). There are reports concerning the sensitivity of *Malassezia* to natural antifungals or anti-*Malassezia* agents. The development of antifungal agents derived from plants produces the anti-*Malassezia* agents that are increasingly efficient. This current study showed that the 96% ethanol extract of the green and yellow kinar (*Kleinhovia hospita* Linn.) leaves did not show any inhibition zones.

Sanjaya et al. (2021) explained the antifungal activity of the plant *Melastoma malabathricum*, which found that the absence of an inhibition zone indicates the inability of the metabolite compounds of the plant in penetrating the cell walls of *Malassezia furfur*. The cell wall matrix of the genus *Malassezia*, such as *Malassezia furfur*, consists of various components in the form of polysaccharides and proteins. It constructs the cell wall of *Malassezia* to be relatively thick and contain a multilaminar ultrastructure and fat so that it is difficult to penetrate (Shibata et al. 2009). *Malassezia furfur* is able to form biofilms to provide protection against other microbes, create safe conditions for the proliferation, act as a barrier from secondary metabolites, such as antifungals, and protect itself from human immune system (Sanjaya et al. 2021). The biofilm consists of a matrix of extracellular polysaccharides, amyloids, DNA, and adhesive fibers that provide permanent adhesion to healthy skin. The area of the biofilms provides a low pH and high concentration of metallic ions which can prevent kinar leaf bioactive compounds from reaching fungal cells (Allen et al. 2015). Yunus & Malik (2019) tested the inhibition of kinar leaf extracts against enteropathogenic bacteria and the results showed the ability of kinar leaf extracts in inhibiting the growth of *Escherichia coli* at a 35% concentratioin ad *Salmonella typhi* at 55% concentration. Kinar leaf extracts are only able to inhibit the growth of bacteria (Stalhberger et al. 2014). The influential factor that might be the cause is the type of solvent used. The solvent used in this study was 96% ethanol, while some previous studies used 70% ethanol to obtain the bioactive compounds needed to inhibit the *Malassezia furfur* because it is more effective than 90% and 96% ethanols in attracting more polar compounds and producing a higher amount of active ingredients (Azis et al. 2014, Kusuma et al. 2018). Stevani (2021) used 70% ethanol as a solvent in the extraction process of kenikir (*Cosmos caudatus* Kunth.) leaves and obtained desirable compounds, such as alkaloids, flavonoids, phenolics, saponins and steroids. Those compounds are known to be able to inhibit the growth of *Malassezia furfur* (Triana et al. 2016, Dirga et al. 2022).

Strength and limitation

In order to treat pityriasis versicolor, it will be important to conduct more study in the area of herbal medicine.to extract more beneficial chemicals, and an appropriate breakdown type and concentration.

CONCLUSION

Due to its activity against the *Malassezia furfur*, kinar (*Kleinhovia hospita* Linn.) leaf ethanol extracts cannot be developed as herbal medicine for the treatment of pityriasis versicolor. It is recommended to conduct further studies on the type of solvent and the appropriate concentration to attract more bioactive compounds.

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Conflict of interest

The authors declare that there was no conflict of interest.

Funding disclosure

The authors received no specific funding for this research.

Author contribution

All the authors contributed to the experimental design of the research, as well as to the acquisition, analysis, and interpretation of the obtained results. Moreover, all the authors contributed to the writing and critical revision of the manuscript.

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Case Report/ Case Report

HYBRID PROCEDURE IN AORTOILIAC BIFURCATION AND FEMORAL LESION

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ABSTRACT

Critical limb ischemia is a condition that threatens the viability of lower extremities and must be treated promptly to avoid major amputation. Revascularization is the most effective treatment method for critical limb ischemia. Revascularization using a hybrid of endovascular and open surgery is a minimally invasive procedure that performs well as the treatment for medically high-risk patients. A hybrid procedure should be considered for patients with high surgical risk. However, there are factors that could compromise its long-term patency, such as critical limb ischemia and diabetes. This study reported a case of a 53-year-old Asian male with history of insulin-dependent diabetes mellitus and long-standing tobacco use, presented with pain in the right leg at rest approximately 30 days prior to admission. Physical examination revealed a low temperature and remarkable non-palpable pulses in the right femoral, posterior tibial, and dorsalis pedis segments. Arteriography with run-offs revealed a long segment of total occlusion from the proximal right common iliac and anterior tibial artery. There was non-significant stenosis in the right popliteal artery. The patient was treated using a combination of percutaneous transluminal angioplasty (PTA) of the right common iliac artery using vascular stent and the Fogarty thrombectomy of the common femoral artery, equipped with an X-ray system and a moveable radiolucent surgical table. The anticoagulant used on the patient during the procedure was heparin. There was no residual stenosis after the procedure on the occlusion along the right common iliac artery to the common femoral artery. In conclusion, multilevel artery occlusive diseases could be treated by hybrid procedure, with shorter hospitalization, less perioperative morbidity, and similar early- and long-term efficacy compared with open revascularization.

***Keywords**: Percutaneous transluminal angioplasty; surgery; thrombectomy; critical limb ischemia; hybrid procedure; tabacco use*

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Highlights:

1. A patient had a long segment of total occlusion to the proximal right common iliac and anterior tibial artery.
2. The treatment is a combination of percutaneous transluminal angioplasty (PTA) using vascular stent and the Fogarty thrombectomy.
3. Hybrid procedure can treat multilevel artery occlusive diseases, with shorter hospitalization, less perioperative morbidity, and similar early- and long-term efficacy.

INTRODUCTION

Critical limb ischemia (CLI) is a manifestation of chronic peripheral arterial disease characterized by complaints of typical ischemic pain during resting. This condition requires immediate management to prevent amputation of the lower extremity. Revascularization is the most effective method in CLI cases, which may be performed using endovascular techniques or open operative techniques (Tendera et al. 2011, Primasari et al 2022).).

Jung et al. (2018) studied 38 patients with CLI related to multistage peripheral arterial disease in 43 limbs that were treated with a one-stage hybrid interventional procedure. The primary and secondary patency rates were at 24 months, and the limb salvage rate was high.

Percutaneous transluminal angioplasty (PTA) and stenting procedures are methods of endovascular revascularization. These are minimally invasive procedures performed in peripheral arterial disease (PAD) cases, such as CLI and intermittent claudication (Norgren et al. 2007). Some advantages of the PTA procedure are low complication rate (0.5% to 4%), technically high successful rate, even in the case of a long occlusion, and clinically acceptable outcomes. PTA procedure is the standard of revascularization in aortoiliac, femoropopliteal, and other below-the-knee blood vessel cases in various vascular intervention centers (Thomas et al. 2015).

In the last few years, hybrid procedure has been developing rapidly. Hybrid technology was first reported in the 1970s (Porter et al. 1973). Since then, most studies have examined common femoral artery endarterectomy or femoro-femoral bypass in combination with endovascular infusion therapy. They reported long-term outcomes comparable to open surgery, with similar or lower morbidity and mortality (Aho & Venermo 2012, Zhou et al. 2014).

The hybrid procedure is a combination of endovascular revascularization with open operative techniques performed simultaneously. A hybrid of open and endovascular procedures can be performed in the same setting or as multi-step procedures. A simultaneous hybrid surgery is performed under fluoroscopy. A staged approach is associated with longer hospital stays, higher costs, groin scarring issues, and multiple surgeries compared to simultaneous hybrid surgery. It is also associated with increased heart risk. The chance of treatment succes can improve if the treatment can be confirmed immediately (Patel et al. 2014, Fekry et al. 2021). CLI patients are generally elderly, frail, and have high mortality. With this in mind, a simultaneous hybrid procedure is a viable and effective treatment for CLI patients with multilevel and high-risk atherosclerosis. Recently, a hybrid of femoral endarterectomy and endovascular therapy for multistage occlusive arterial disease was established. Concurrent common femoral artery endarterectomy (CFE) and endovascular therapy (EVT) for iliac or superficial femoral artery lesions has been proposed as a valid alternative technique in opening the aortofemoral or infrainguinal bypass grafts (Nelson et al. 2002, Patel et al. 2014). The hybrid procedure has begun to be a choice along with the development of vascular imaging system in the operating room and the development of endovascular expertise. Some types of re-vascularization in hybrid procedures can be divided into bypass surgery or thromboendarterectomy combined with catheterization-based endovascular interventions to increase inflow and outflow in the vessels (Norgren et al 2007).

This study reported a case of a CLI hybrid procedure performed on a 53-year-old male patient with clinical findings of right leg pain for approximately 30 days, with a physical examination that revealed a low temperature and remarkable non-palpable pulses in the right femoral, posterior tibial, and dorsalis pedis arteries.

CASE REPORT

A patient, Mr. S, a 53-year-old male patient from Tuban, East Java, Indonesia, was admitted to Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, to carry out a catheterization of the heart and right leg blood vessels. The patient complained right leg pain since the last 30 days with left chest pain two months before being hospitalized. The chest felt burning sensation, especially with activities and improved with rest. Past medical history showed that the patient had risk factors, i.e. hypertension for 5 years, type 2 diabetes mellitus for 5 years, newly discovered dyslipidemia, and heavy smoking for 20 years. The patient claimed to visit the doctor regularly and take medication regularly in the last 5 years.

The physical examination showed good general condition, sound mental health, blood pressure of 120/80 mmHg, regular pulse of 70 per minute, respiration rate of 18 per minute, and afebrile condition. The head and neck examination did not show anemia, jaundice, cyanosis, and dyspnea. The jugular venous pressure (JVP) did not increase. The physical examination of the heart showed an apex beat (ictus cordis) on the left intercostal space (ICS) VI along the midclavicular line, single S1 and S2 heart sound regularity, while no murmur, gallop, or extrasystole were obtained. The pulmonary examination showed vesicular breath sounds in both lung fields, while rheumatism and wheezing were not found. The examination of the abdomen showed suppleness and normal bowel sounds, meanwhile the liver and spleen were not palpable. Both upper extremities were warm, dry, and red, with no presence of edema. The right lower extremity showed reddish skin from the knee to the toe tips. Palpation did not show from the right femoral artery to the right dorsalis pedis artery. The Ankle Brachial Index (ABI) values were 0.58 in the right lower extremity and 0.9 in the left lower extremity.

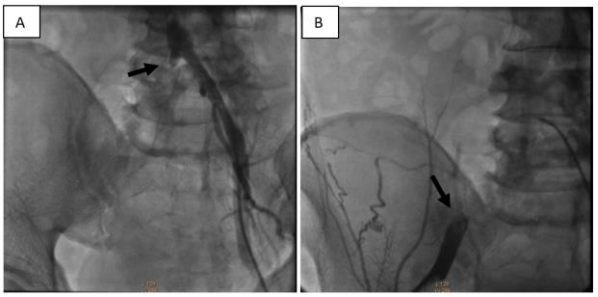

Figure 1. (A) Results of an angiography examination showing total occlusion of the right proximal common iliac artery (arrow) from an anterior approach; (B) total occlusion of the right external iliac artery (arrow) seen from a retrograde approach

The admission electrocardiogram (ECG) examination showed a sinus rhythm of 75 beats per minute, normal axis, and inferior occlusion myocardial infarction (OMI). The chest X-ray examination showed a cardiomegaly, with cardio thoracic ratio (CTR) of 59%, and lung conditions within normal limits. The laboratory test showed a hemoglobin (Hb) of 13.0 g/dl, a leukocyte count of 7,900 cells/µL, a platelet count of 290,000/µL, a serum glutamic-oxaloacetic transaminase (SGOT) of 30 U/L, a serum glutamic pyruvic transaminase (SGPT) of 30 U/L, a blood urea nitrogen (BUN) of 10 mg/dL, a streptokinase (SK) 1.0 mg/dL, a serum sodium concentration of 135 mmol/L, a potassium level of 4.3 mmol/L, a chloride level of 100 mmol/L, a random blood sugar level of 180 g/dL, a non-reactive hepatitis B surface antigen (HbsAg), a non-reactive hepatitis C virus (HCV) antibody, a partial thromboplastin time (PPT) of 10.6 seconds, and an activated partial thromboplastin time (aPPT) of 27.1 seconds. The echocardiographic examination showed that the valves had no abnormalities, the dimension of the four heart chambers was within normal limits, the decrease in left ventricular systolic function was 54%, and thrombus and intracardiac vegetation were not present. The segmental analysis of the left ventricle showed a ventricular hypokinesia and a concentric left ventricular hypertrophy (LVH). The vascular Doppler ultrasound showed a decreased peak of systolic rate of the right femoral artery and right popliteal artery, while no thrombus was found in the evaluated arterial and venous system. Non-specific lymphadenopathy was found in the inguinal region and right proximal femur.

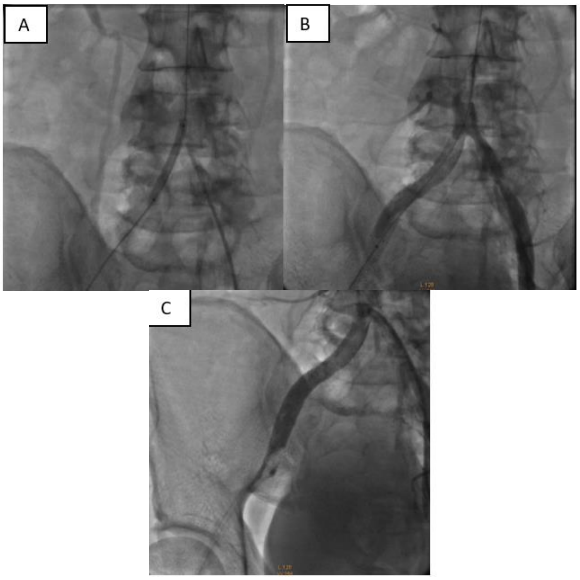

Figure 2. (A) The process of placing and inflating balloons in the right common iliac artery up to the right common femoral artery. (B) The process of placing and expanding vascular stent in right common iliac artery. (C) Multiple thrombus observed along the right common iliac artery up to the right common femoral artery

The patient underwent inferior lower extremity PTA procedure and coronary angiography with the following results. In the right lower extremity, the chronic total occlusion (CTO) was proximal to the common iliac artery. A non-significant stenosis of 40% was found in the popliteal artery. CTO was also seen in the anterior tibial artery. In the left lower extremity, a non-significant common iliac artery stenosis of 20% and a non-significant internal iliac artery stenosis of 40% were found. Non-significant stenosis was also present in the common femoral artery. The distal part of the common femoral artery was not evaluated. The coronary angiography results indicated a single-vessel disease (SVD).

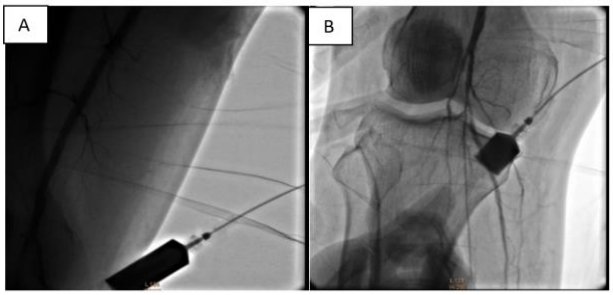

Figure 3. (A) A non-significant stenosis of 40% seen in the popliteal artery; (B) CTO observed in the anterior tibial artery.

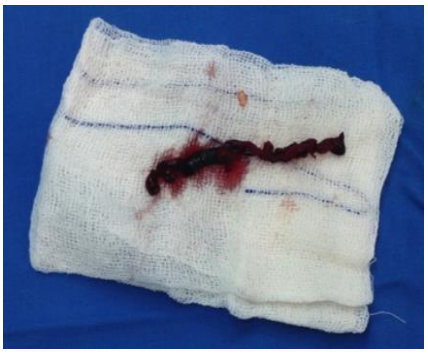

Figure 4. Thrombus after the Fogarty thrombectomy procedure

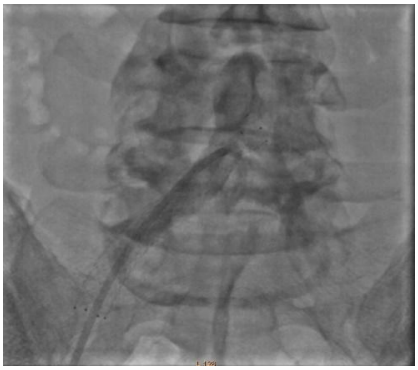

Figure 5. Vascular stent migration appears more proximal in the aorto-iliac bifurcation.

DISCUSSION

This report provides an overview of the management of CLI with complex and multilevel lesions using a minimally invasive approach, the hybrid procedure. The hybrid procedure in CLI cases has been developed and until now the success rate has reached up to 93%-100% (Kwa et al. 2011). The most common typical location of atherosclerosis is the bifurcation of the common femoral artery. If atherosclerosis occurs in common iliac artery, the use of endovascular method in the revascularization is more appriopriate. The method has a high success rate, a lower rate of complications, and a better long-term prognosis. However, the presence of lesion in common femoral artery poses many challenges if endovascular method is performed. The first reason is that the lesion often involves both branches of the common illiac artery, the internal iliac and external iliac. In addition, the placement of balloon angioplasty in common femoral artery may cause closure of the deep femoral artery if the guidewire is placed on the superficial femoral artery. The second reason is that the common femoral artery lesions in the groin is strongly affected by high and continuous mobility or movement, so that any installed stent will be vulnerable to fracture complications.

The lesion in this reported case belonged to the aortoiliac occlusive disease (AIOD) lesion type. AIOD lesion often involves lesions in distal sites and common femoral arteries. It also has a high success rate of blood vessel patency (80%-83%) if a combined procedure of angioplasty and stent placement in illiac artery (as the first line therapy) and arterial reconstruction in the distal part (hybrid) is performed (Mustapha et al. 2014).

Diagnosis

Critical limb ischemia (CLI) is considered as the most severe in the peripheral arterial disease (PAD) pattern. It is associated with a high risk of major amputation, death, and cardiovascular events (Murabito et al. 2002, Uccioli et al. 2018). CLI is a clinical manifestation of perpiheral arterial disease (Rutherford II, Fontain III classifications) characterized by resting ischemic pain and accompanied by ischemic lesion due to blockage of peripheral arteries. This condition is chronic and must be distinguished from acute limb ischaemia (ALI) (Norgren et al. 2007, Tendera et al. 2011).

The mortality rates of reported cases were 20% within 6 months after the diagnosis and 50% at 5 years (Stoyioglou & Jaff 2004, Adam et al. 2005). This excessive mortality may be related to the systemic cardiovascular diseases, including coronary artery disease and cerebrovascular arterial disease (Caro et al. 2005, Steg et al. 2007). Furthermore, CLI is associated with peripheral complications, such as ulceration, gangrene, infection, and a high risk of lower limb amputation estimated in non-treatable patients (Norgren et al. 2007, Abu Dabrh et al. 2015). Patients with CLI have three times higher risk of myocardial and stroke infarction (Mustapha et al. 2014). The incidence of CLI reaches 500 to 1000 per million people per year, with the highest frequency occurrs in smokers, elderly individuals, and those with diabetes mellitus. The amputation in CLI cases ranges from 5% to 20% in patients who cannot undergo revascularization, those with neurological disorders and are unable to mobilize. In contrast, the success rate of CLI cases that can be revascularized can reach 90%.

The establishment of CLI diagnosis is based on history, physical examination, and investigations. Patient history reveals pain, even during a resting position, that lasts for more than two weeks. Physical examination shows ischemic or gangrene lesions, which indicate chronic vascular occlusion. The Ankle Brachial Index (ABI) examination can also be used in the diagnostic process. CLI is diagnosed if the ABI examination produces a ratio less than 0.9 and absolute ankle pressure less than 50-70 mmHg. Another examination is transcutaneous oxygen pressure of less than 30 mmHg, which can be used not only for diagnosis and prognosis, but also for determining amputation level. Other investigations that can be used are exercise tests (treadmill test), Doppler Ultrasonography (DUS), Computed Tomography Angiography (CTA), and Magnetic Resonance Angiography (MRA) (Tendera et al. 2011).

The classification of lesions according to the 2007 Trans-Atlantic Inter-Society Consensus Document on Management of Peripheral Arterial Disease (TASC II) had been subjected to several revisions as compared to the initial version. The changes were based on technological progress. However, the basic principle of the classification has not changed. The basic principle is based on revascularization techniques. Type A lesions are lesions that can provide remarkable outcome if endosvascular revascularization is performed. Type B lesions are lesions with a choice of first-line revascularization by means of endovascular method, and, if needed, can use open operative techniques in the same anatomical segment. Type C lesions may have a good prognosis in the long term if revascularization is done using open operative techniques. This type of

endovascular technique in type C is only used in patients at high risk without adequate distal vein or vascularization. Type D lesions do not produce good results if revascularization with endovascular techniques is performed (Norgren et al. 2007, Klein et al. 2014).

The Trans-Atlantic Inter-Society Consensus Document on Management of Peripheral Arterial Disease (TASC) has provided management guidelines according to the classification of lesions. However, patients with CLI often have multiple anatomical lesions, so the use of lesion classification is sometimes difficult to apply. The management of revascularization is still based on a comprehensive evaluation of individual patient and the characteristics of the lesions and target lesions. In the patient of this case study, the angiography revealed type C vascular lesions involving the common iliac artery, external iliac artery, common femoral artery, and internal iliac artery.

Management

According to the 2011 European Society of Cardiology guidelines, the initial management of CLI, if possible, is immediate revascularization (class of recommendation (COR) I; level of evidence (LOE) A). The revascularization technique recommended as the first line is the endovascular technique (COR IIB; LOE B) (Tendera et al. 2011). The TASC II data showed that the management of 2,222 iliac artery cases (76% intermittent claudication cases and 24% CLI cases) using angioplasty procedures had technical success rate of 96%. Follow-ups after one, three, and five years show that the success rates of blood vessel patency managed with angioplasty were 86%, 82% and 71%. Other research on 505 aortoiliac cases managed with angioplasty and/or stent procedures showed that the success rate after 9 years was 98%, with only 0.5% mortality rate after 30 days (Norgren et al. 2007, Scheinert & Schmidt 2011).

In general, endovascular procedure is not performed as a prophylaxis procedure in asymptomatic patients. Data showed that until now there has been no other method besides the installation of stents to increase blood vessel patency during angioplasty (Scheinert & Schmidt 2011). The achievement of optimal blood vessel patency after angioplasty is higher if it is performed on the common iliac artery. The patency will be lower if the angioplasty is carried out more distally, especially if the lesion is long, multiple, and wide, and the patients suffer from diabetes and kidney failure (Stone & Cambria 2005).

The use of drug-eluting balloons (DEB) is promising. However, data supporting its use are still very limited in general population. The primary goal of stenting is preventing residual stenosis, extensive recoil, and dissection, while also improving long-term vascular patency. The installation of stent should avoid areas with sharp angles, such as the groin and knee, although special stents have now started to develop. In addition, it is not recommended for vascular stents to be installed in sites that can be used as a ground zone in the implementation of bypass procedures (Scheinert & Schmidt 2011, Zou et al. 2012). The strategy of installing primary stenting on lesions of the external iliac arteries using self-expandable stent is more recommended than provisional stenting in terms of the risk of dissection and elastic recoil (Stone & Cambria 2005).

Choosing which strategy to penetrate the lesions in CTO cases with anatomic variations greatly affects the level of success. The determination of intervention strategies using retrograde or antegrade techniques is based on the lesion configuration and the presence or absence of hibernating lumen (HL). The HL is a patent arterial lumen segment that is located between two CTO caps and has a heterogeneous calcium distribution. If HL is present, the CTO cap needs to be identified to determine the approach of lesion penetration, whether antegrade or retrograde. The next step is to determine the configuration of proximal and distal CTO cap surface, which can be concave or convex (Figure 5). In general, the intervention approach follows the concave form of the CTO cap surface, so that it can increase the likelihood of success in penetrating CTO lesions (Hardman et al. 2014).

Aortoiliac occlusive disease (AIOD) often involves lesions on the distal side and common femoral artery. It has a high success rate of blood vessel patency (80%-83%) if a hybrid procedure of angioplasty and arterial stenting (first line), as well as simultaneous operative reconstruction of the distal artery, is performed. AIOD lesion indicates a need of hybrid procedure if it extends into the common femoral artery, if there is a long occlusion lesion in the external iliac artery, and if a stent is to be installed. Hybrid procedure is used to correct vascular inflow and outflow. In AIOD lesion, inflow is corrected by performing endovascular procedure in the aortoiliac and open surgery in the distal artery, especially along the common femoral artery to the distal part. In the patient of this case study on hybrid procedure, the PTA stent was installed in the right common iliac artery, while the Fogarty thrombectomy

was performed on the distal part of the common femoral artery.

Complications

Percutaneous transluminal angioplasty (PTA) in the iliac artery, whether or not accompanied by stents, is a safe procedure, although complications can still occur afterwards. Complications of PTA in iliac arteries, although rare (7.9% to 23.7%), can cause significant disability (Ahmed et al. 2005). Some complications are classified into two, those related to the procedures performed and those related to the installed equipment.

Complications related to PTA include complications in the entry point of the procedure, dissection, rupture or perforation, aneurysm/pseudoaneurysm formation, distal embolization, and closure of internal iliac artery. Complications associated with stents attached are stent thrombosis, stent migration, stent crush, and infection. In this reported case, the complication that occured was the migration of the stent, which moved closer to the proximal part of the right aortoiliac bifurcation.

Prognosis

A study by Nelson et al. (2002) showed that stenting procedures in external iliac arteries combined with open surgery on common femoral arteries provided satisfactory outcome, reaching 100% success rate technically and hemodynamically at the beginning after the procedure. After a year, the success rate of the vascular patency reached 84% and 97%. The technical success occurs if the residual stenosis found to be less than 30% and without complications, such as embolism or thrombosis. Whereas, the hemodynamic success arises from the Ankle Brachial Index (ABI) value increase of more than or equal to 0.01. Another study by (Cotroneo et al. 2007) also provided similar results, with the success of vascular patency after two years reached 79% and 86%.

Strength and limitation

By finding critical limb ischemia in the patient in this study who has a history of insulin-dependent diabetes and long-term tobacco use, this case report can contribute to recent studies on surgery. This study also provides additional evidence that efficient therapies using open surgery and a hybrid of endovascular procedures, revascularization

CONCLUSION

A 53-year-old male patient was reported with a history of hypertension risk for 5 years, type 2 diabetes mellitus for the past 5 years, newly discovered dyslipidemia, and heavy smoking for 20 years. The patient was admitted to the hospital with complaints of right leg pain since the last 30 days, accompanied by left chest pain for two months before admission. Chest burning occurred especially during activities and improved with rest. The Ankle Brachial Index (ABI) values were 0.58 in the right extremity and 0.9 in the left extremity. PTA procedure on the right lower extremity revealed a CTO that was proximal to the common iliac artery, a non-significant stenosis of 40% in the popliteal artery, and a CTO in the anterior tibial artery. A hybrid procedure using the PTA stent and the Fogarty thrombectomy was performed simultaneously with satisfactory results.

Acknowledgment

I especially thank the patient (Mr. S) for agreeing to publish this case report. I am very grateful to Dr. Soetomo General Academic Hospital team, the peer reviewers for their time and comments, and also the journal editorial team for publishing this case report.

Patient's consent for publication

The patient signed the informed consent form and agreed that this case report is published.

Conflict of interest

I had no conflict of interest in this research.

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Author contribution

Yudi Her Oktaviono wrote, revised, and edited the methodology and manuscript, and also collected the sources used.

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Review Article

CLINICAL IMPORTANCE OF SARCOPENIA AND HOW IT IMPACTS ORTHOPEDIC-TRAUMA PATIENTS AND THE SURGICAL OUTCOMES

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ABSTRACT

Sarcopenia is a condition of low muscle strength, mass, and low physical performance that is affected by age (primary sarcopenia) and one or combination of systemic diseases, physical inactivity, and insufficient intake of energy (secondary sarcopenia). This condition affects one in ten healthy adults aged ≥60 years. There are two widely used criteria to diagnose sarcopenia, the Asian Working Group for Sarcopenia (AWGS) and the European Working Group on Sarcopenia in Older People (EWGSOP). These working groups created algorithms to facilitate the diagnosis. Establishing the diagnosis is crucial because it has deleterious impacts on the patients, such as increasing risks of mortality, morbidity, falls, complications during and after surgery, disability, prolonged hospitalization, and fractures. Sarcopenia is considered an independent mortality risk. It is paramount for physicians to assess this condition before treating the patients because it can predict the risk and plan better treatment options to achieve better outcomes. Early assessment is crucial, even for surgeons. Sarcopenia also negatively impacts patients who had surgery. Up to 44% patients who underwent orthopedic trauma surgery had sarcopenia. The high percentage was affected by the increased risk of falls and fractures. On that account, this condition needs to be treated. The main treatments for this condition are exercise and adequate nutrition intake. The recommended exercise as a first-line treatment is resistance or strength training. Overall, knowledge on sarcopenia can prepare clinicians and surgeons in anticipating the implications of sarcopenia.

Keywords: *Physical performance; muscle strength; sarcopenia; elderly; health risk; human mortality; muscle mass*

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Highlights:

1. Sarcopenia can cause deleterious effects on patients.
2. The Asian Working Group for Sarcopenia (AWGS) and the European Working Group on Sarcopenia in Older People (EWGSOP) provide the most widely used criteria to diagnose sarcopenia.
3. Knowledge of sarcopenia should be a trigger for clinicians and surgeons to evaluate the treatment plan and anticipate the implications experienced by the patients.

INTRODUCTION

The term "sarcopenia" to refer the loss of skeletal muscle mass was first coined by Irwin Rosenberg in 1989 in a meeting at Albuquerque, New Mexico, United States of America. He noted that lean body mass is the most affected part of the body that declines as humans are aging. It affects human mobility, ambulation, nutritional intake, health status, and even breathing. The etymology of the term "sarcopenia" is that it originated from Greek words "*sarx*" that means flesh (muscle) and "*penia*" that means loss (Rosenberg 1997). Yet, the operational definition was still lacking. In 2010, a working group focused on geriatric and sarcopenia, the European Working Group on Sarcopenia in Older People (EWGSOP), stated the three criteria to determine the diagnosis of sarcopenia, which are muscle mass, strength, and physical performance assessed respectively (Cruz-Jentoft et al. 2010). However, this same group revised the diagnosis criteria in 2018, with loss of muscle strength is assessed first, then followed by the physical performance and muscle mass (Cruz-Jentoft et al. 2019a).

Since the term proposed by Rosenberg (1997a), sarcopenia was suspected to be caused by the aging of human. Statistics showed that sarcopenia simultaneously increases with the increasing age of people (Shafiee et al. 2017). However, numerous studies on sarcopenia revealed other contributing factors, such as anorexia, inflammation, hypogonadism, sedentary lifestyle, vitamin D deficiency, insulin resistance, genetic causes, and cancer (Friedman et al. 2015, Anker et al. 2016). These factors could cause a decrease in the quality of life, disability, and higher risk of death.

It is essential to predict the risk of mortality and morbidity of a person who will have a major surgery. The prediction might influence the clinician and/or surgeon to choose the treatment/surgical plan. Patients affected by sarcopenia are more likely to have crucial physiologic stress during operation, surgical complications, and a higher risk of death during and/or post-operation (Friedman et al. 2015). Another issue is that prolonged hospital stay is required, therefore more costs are spent (Wahlen et al. 2020).

The presented description of sarcopenia clarifies that its impacts are extensive, including in patients who underwent orthopedic-trauma surgery. A retrospective study by Ji et al. (2014) showed that orthopedic patients who underwent surgery had a higher sarcopenia prevalence a half time more than non-orthopedic surgery patients. The increased risk of falls in elderly is in conjunction with the increasing risk of fractures in people with sarcopenia, such as fragility fractures of the hip (FFH) (Landi et al. 2012, Laubscher et al. 2020). These studies showed that sarcopenia negatively influenced and increased the morbidity of orthopedic patients. Studies has showed the negative impacts of sarcopenia on patients, especially the elderly and orthopedic trauma patients, and the consideration of its risks and implications by clinicians and surgeons to achieve a better outcome of treatment. This narrative article focuses on reviewing the deleterious impacts and consequences of sarcopenia to the orthopedic trauma patients along with the surgical outcome. The study of sarcopenia and its impacts on orthopedic trauma patients has not been discussed in any Indonesian journal, although has been widely known and discussed in many papers published by international journals. For that reason, hopefully this narrative review would bring an overview and new insights to the readers and then spark new ideas for other research.

OVERVIEW

Sarcopenia is described as a progressive decrease in skeletal muscle mass and strength caused by increasing age, with the burden of physical disability, falls, and mortality (Rosenberg 1997). The EWGSOP stated in 2010 that the diagnosis of sarcopenia is established using an assessment of low muscle mass and function (performance and/or strength). However, the 2010 EWGSOP did not mention any age-related decrease in muscle mass and function (Cruz-Jentoft et al. 2010). In 2018, the EWGSOP revised the consensus guideline (the so-called EWGSOP2) by putting low muscle strength in the forefront of the diagnostic criteria, meaning at least low muscle strength is required to suspect sarcopenia. The criteria also includes the quantity/quality of low muscle and/or low physical performance (Cruz-Jentoft et al. 2019a).

Although the 2010 EWGSOP criteria for sarcopenia triggered extensive research on sarcopenia, it was not adequate for Asian people who have different anthropometrics, cultures, and lifestyles. These differences influence researchers in Asia to establish another working group called the Asian Working Group for Sarcopenia (AWGS) in 2014. The AWGS updated their consensus on sarcopenia diagnosis and treatment in 2019. According to the AWGS, sarcopenia is defined as the loss of muscle mass along with a loss of strength and/or diminishing physical performance that is related to aging, without any reference to comorbidity. However, younger people (not included in the categorization of older people in each country) who have low muscle mass and/or function accompanied by comorbidity, such as paralysis or cachexia, are not diagnosed with sarcopenia, although early identification of the causes is still needed (Chen et al. 2020).

The EWGSOP2 divides sarcopenia into two categories. The first category primary sarcopenia, which is related to aging process. On the other hand, the second category is not age-related. Secondary sarcopenia occurs due to systemic diseases, physical inactivity (i.e. sedentary lifestyle), and/or insufficient intake of energy. The AWGS criteria only considers sarcopenia as an age-related process (Cruz-Jentoft et al. 2019a, Chen et al. 2020). The primary sarcopenia is caused by the aging process associated with physiological changes in humans. However, this category could overlap with other diseases that simultaneously occur as people aging, such as osteoarthritis (OA) or cancer that causes cachexia (inadequate intake of energy). The diseases might cause other problems, such as physical inactivity. These descriptions clarify that sarcopenia has multiple factors that interact with each other (Cruz-Jentoft et al. 2019a).

Tarantino et al. (2015) described that many factors contributed to the patophysiology of sarcopenia, i.e. physiological changes in cellular turnover, denervation of muscle fibers, inflammation (related to the increase of interleukin and tumor necrosis factor), muscle protein degradation, reduction of hormone synthesis modulation, physical inactivity, inadequate intake of nutrition that alters metabolism, and apoptosis. The physiological changes in muscle cell levels can be seen when people get older. Those changes can cause the loss of muscle mass, such as adipose tissue accumulation around muscle fibers, the reduced anabolic influence of endocrine system, and even a drop of motor neurons numbers associated with the reduced maximal motor unit firing rates (Frontera et al. 2012). Aging process include the reduction of testosterone and growth hormone concentration in plasma at 7% every four years and 14% per decade respectively, which can result in negative anabolic effect in the endocrine system. These two hormones are

essential in increasing muscle protein synthesis because they act as powerful anabolic agents (Tieland et al. 2018).

As people get older, there is a rise in subclinical inflammation in the muscle that increases the muscle catabolism. This inflammation may be partially overcome by resistance training. The elevation of inflammatory markers, such as interleukin-6 (IL-6), will result in progressive loss of muscle mass and quality (Tieland et al. 2018). Other problems may also occur, i.e. the rise of circulating myostatin levels that is counter-regulative to muscle mass, the decline of myosin concentration that affects muscle contractility, the changes of lifestyle to be more sedentary that is mostly found in elderly and further decrease the myosin concentration (Frontera et al. 2012, Tieland et al. 2018).

Insufficient nutritional intake also plays an important role in preventing sarcopenia. Vitamin supplementation is used as treatment strategy for sarcopenia. Vitamin D works as calcium homeostasis regulator that maintains the development of skeletal muscle function. Those who have deficiency of Vitamin D have up to 3.3 times higher risk to develop sarcopenia (Yoo et al. 2021).

A research by Shafiee et al. (2017) studied the prevalence of sarcopenia around the world using the AWGS, EWGSOP, and International Working Group on Sarcopenia (IWGS) criteria. The overall estimates of sarcopenia prevalence were 10% in women and 10% in men from a total of 58,404 healthy adults aged ≥60 years, with a higher tendency of non-Asians to be sarcopenic than Asians in both genders (19% vs 10% in men, 20% vs 11% in women). The reason behind these numbers was associated with differences in body anthropomorphism, dietary aspects, daily life activity, and cultural background of Asian and non-Asian. Other factors correlated with developing numbers of sarcopenia included diabetes, hypertension, and dyslipidemia (Shafiee et al. 2017, Chen et al. 2020).

Another study by Petermann-Rocha et al. (2022) revealed that statistically the use of the EWGSOP2 criteria resulted in numbers, in which men were more affected than women (11% vs 2%). Whereas, the use of the AWGS criteria showed that women had a higher sarcopenia prevalence than men (17% vs 12%). These differences appeared because different criteria were used, as happened in numerous studies. A study conducted by Widajanti et al. (2020) in Surabaya, Indonesia, showed that of 308 participants aged ≥60 years, the prevalence rate of sarcopenia was 41.8% (129 participants) and one in five sarcopenic participants had severe sarcopenia. A study by Ji et al. (2014) showed that up to 44% orthopedic patients who underwent surgery reported sarcopenia occurrence.

Many research on sarcopenia using the EWGSOP2 and the 2019 AWGS (revised update of the 2014 AWGS) as the diagnostic criteria. The diagnosis of sarcopenia according to the EWGSOP and the AWGS is as follows.

The operational definition of sarcopenia differs from probable sarcopenia and severe sarcopenia. The criteria to establish the diagnosis include the quality/quantity of low muscle, muscle strength, and low physical performance. The criteria are checked sequentially. Probable sarcopenia is identified if only the first criteria is apparent. Sarcopenia is identified if probable sarcopenia is accompanied by low muscle quantity/quality or low physical performance. Severe sarcopenia is confirmed if all of the three criteria exist. The EWGSOP made a method for easier screening and diagnosing of sarcopenia, i.e. find cases-assess-confirm-severity (FACS) (Cruz-Jentoft et al. 2019b)

The causes of sarcopenia can be found using a screening tool, i.e. a questionnaire that includes strength, assistance, rise from a chair, climb stairs, and falls (SARC-F) as seen in Table 1. This screening tool has five questions, with 0 to 2 points for each question. A score of ≥4 is considered symptomatic and needs further assessment to identify the sarcopenia category. A score of <4 is considered healthy.

Table 1. SARC-F questionnaire and scoring

Criteria	Questions	Score
Strength	How much is the difficulty to lift/carry 10 pounds (4.5 kilograms) weight?	0=no difficulty 1=some difficulty 2=a lot of difficulty
Assistance	How much is the difficulty to walk across a room and whether the use of aid or help is needed?	0=no difficulty 1=some difficulty 2=a lot of difficulty, use aids, or unable to do without personal help
Rise	How much is the difficulty to transfer from a chair or bed and whether the use of aid or help is needed?	0=no difficulty 1=some difficulty 2=a lot of difficulty, use aids, or unable to do without personal help
Climb	How much is the difficulty to climb a flight of 10 steps?	0=no difficulty 1=some difficulty 2=a lot of difficulty
Falls	How many falls are experienced for the past one year?	0=no falls 1=1–3 times falls 2=>3 times falls

The assessment of sarcopenia is conducted by measuring muscle strength. The parameters used for this measurement are the handgrip strength and chair stand test. The handgrip strength is assessed using a validated and calibrated handheld dynamometer, such

as the Jamar dynamometer. The chair stand test assessed the leg muscle strength and endurance, especially the quadriceps muscle. The test counts how many times a patient is able to stand-sit over 30 seconds intervals. The assessment is displayed in Table 2.

Table 2. Muscle strength assessment

Test	Men	Women
Hand grip strength	<27 kg	<16 kg
Chair stand test	>15 seconds for five rises	

If the handgrip and chair stand test points are low, then a probable sarcopenia is identified. The next step is to confirm the sarcopenia by estimating the muscle quantity or mass using various techniques and categorizing the results based on the height and/or body mass index (BMI). The measured muscle mass/quantity can be the skeletal muscle mass (SMM), appendicular (upper and lower limbs) skeletal muscle mass (ASM), or any specific muscle group or area located cross-sectionally. The golden standard tools for the assessment are magnetic resonance imaging (MRI) and computed tomography (CT) scans. Other tools that can be used are bioelectrical impedance analysis (BIA) and dual-energy X-ray absorptiometry (DXA). Table 3 exhibits the cut-off points.

Table 3. Muscle mass/quantity assessment

Adjustment	Men	Women
ASM	<20 kg	<15 kg
ASM/height^2	<7.0 kg/m^2	<5.5 kg/m^2

After the diagnosis sarcopenia is confirmed, then its severity is assessed. However, the severity cannot be assessed in some patients who have comorbidity, such as dementia, gait disorders, and balance disorders. The assessment measures the physical performance (mobility of whole-body function) by evaluating the short physical performance battery (SPPB), timed up and go test (TUG), and 400 m walk test. Table 4 shows the cut-off points. If a test result is below the cut-off point, then severe sarcopenia is identified. The severity gets worse when the other test results are below the cut-off points.

The operational definitions of sarcopenia in the AWGS and the EWGSOP2 are almost the same. The differences are that the AWGS opposes to include the use of comorbidity factor that causes muscle wasting in the criteria and also retains the age-related cut-off depending on the categorization of "older people" in each country. Loss of muscle mass along with loss of its strength and/or diminished physical performance is in conjunction with age-related condition without any reference to any comorbidity (Chen et al. 2020).

Table 4. Physical performance assessment for the severity of sarcopenia

Test	Instruction	Cut-off points
Gait speed	Speed measure of 4 m walking	≤0.8 m/seconds
SPBB	Cumulative assessment of balance, gait speed, and chair stand test	≤8 points
TUG	Rise from chair → walk 3 m → turn, walk back, and sit	≥20 seconds
400 m walk test	Walk 20 laps of 20 meters (total 400 meters) as fast as possible, and up to two rest stops are allowed	Non-completion or ≥6 minutes for completion

The algorithm of sarcopenia diagnosis in the AWGS almost has a similar pattern as the EWGSOP2. The algorithm is made from case finding (screening), assessment, diagnosis, and severity grading. There are two different algorithm methods to determine the occurrence of sarcopenia in the context of different place-based settings, although the algorithms will eventually be connected at one point. The methods are divided into the algorithm for primary health care or community preventive service settings and the algorithm for acute to chronic health care or clinical research settings.

There are three ways to identify sarcopenia, i.e. the SARC-F questionnaire as used in the EWGSOP2, the SARC questionnaire plus calf circumference (SARC-CalF), and calf circumference (CC) only. The levels of sensitivity of these three ways from low to high respectively are the SARC-F, CC, and SARC-CalF. The SARC-F questionnaire has been discussed in the EWGSOP2 and recommended as case finding questionaire. However, the AWGS recommended CC more than the SARC-F because it has higher sensitvity and specificity in predicting sarcopenia. The CC cutoffs in the AWGS are <34 cm for men and <33 cm for women.

The top recommendation for screening in the AWGS is the SARC-CalF because it yields a cumulative score of the other two ways, therefore it is superior than CC or SARC-F alone. This recommendation is also supported in a validation study by Barbosa-Silva et al. (2016) who recommended to improve the SARC-F screening in clinical practice by associating it with CC. The CC score of the SARC-CalF in this validation study is 10 points for ≤34 cm CC in men and ≤33 cm CC in women (Table 5). If the SARC-CalF score is ≥11 points, then it is recommended to assess the muscle strength or physical performance.

Table 5. SARC-CalF questionnaire and scoring assessment of the AWGS

Criteria	Questions	Score
Strength	How much is the difficulty to lift/carry 10 pounds (4.5 kilograms) weight?	0=no difficulty 1=some difficulty 2=a lot of difficulty
Assistance	How much is the difficulty to walk across a room and whether the use of aid or help is needed?	0=no difficulty 1=some difficulty 2=a lot of difficulty, use aids, or unable to do without personal help
Rise	How much is the difficulty to transfer from a chair or bed and whether the use of aid or help is needed?	0=no difficulty 1=some difficulty 2=a lot of difficulty, use aids, or unable to do without personal help
Climb	How much is the difficulty to climb a flight of 10 steps?	0 = no difficulty 1=some difficulty 2=a lot of difficulty
Falls	How many falls are experienced for the past one year?	0=no fall 1=1–3 times falls 2=>3 times falls
Calf Circumference	What is the measurement of the right calf circumference while the legs are relaxed and feet are 20 cm apart	Male <34 cm=10 points Male ≥34 cm=0 point Female <33 cm=10 points Female ≥34 cm=0 point

The next step to diagnose probable sarcopenia is assessing the patients' hand grip strength or physical performance. Early adjustment to the diet and exercise can be done before the diagnosis of confirmed sarcopenia is established. The cutoff points in the AWGS is slightly different than the EWGSOP2. Table 6 shows the cutoff points of the assessment. After the assessment, the next step is confirming the diagnosis by referring the patients to acute to chronic health care or clinical research settings.

Table 6. The AWGS assessment of muscle strength and physical performance

Test	Men	Women
Hand grip strength	<28 kg	<18 kg
Chair stand test	≥12 seconds for five rises	

Other than the SARC-F/CC/SARC-CalF tests, there are some clinical conditions that should be included in the screening, such as functional decline, unintentional weight loss, depressive mood, malnutrition, and comorbidity with chronic conditions including heart failure. The patients that are referred to these care settings will not be retested on the physical performance. Only ASM examination is used in this setting to establish the sarcopenia diagnosis and grade its severity. Sarcopenia is confirmed if the patient has low ASM plus low muscle strength or low physical performance. Whereas, severe sarcopenia is confirmed if the patients get low score on all ASM, muscle strength, and physical performance. Table 7 shows the cutoff points of the severity assessment.

Table 7. Physical performance test and ASM test in acute to chronic health care or clinical research settings

Test		Instruction	Cut-off points
Physical Performance	6 m walk	Speed measure of 6 meters walking	<1 m/second
	SPBB	Cumulative assessment of balance, gait speed, and chair stand test	≤9 points
	Chair stand test	5-time chair stand test	≥12 seconds for five rises
ASM	Dual-energy X-ray Absorptiometry	Men <7.0 kg/m^2 Women <5.4 kg/m^2	
	Multifrequency BIA	Men <7.0 kg/m^2 Women <5.7 kg/m^2	

Sarcopenia is a deleterious prognostic factor for patients. The aftermath of sarcopenia in the elderly is detrimental as it is considered as an independent mortality risk in a cohort study by Arango-Lopera et al. (2013). A prospective cohort study called ilSIRENTE conducted by Landi et al. (2012) with a total of 260 participants aged >80 years showed that one out of four people was diagnosed with sarcopenia and they were more likely to have impaired cognitive function, vision impairment, hearing impairment, and also higher prevalence of diabetes, stroke, and depression. The risk factors also applied to surgical outcomes. Understanding this risk factors is of the utmost importance to avoid complications during and after surgery, mortality, morbidity, longer hospitalization, and even financial problems. Therefore, surgeons must be able to anticipate the risk factors through treatment methods according to the risk assessment of sarcopenic patients, which will improve the surgical outcomes (Friedman et al. 2015).

Bone and muscle are interconnected tissues that work for each other. When there is an issue (e.g. sarcopenia) in each of these tissues that affects the muscle as

people get older, there will be consequences to the bone. Sarcopenia could coexist with osteoporosis (Tarantino et al. 2015). It also had been presented in a prospective study by Petermann-Rocha et al. (2021) who showed that the risk of developing osteoporosis was 1.3 times higher in men with pre-sarcopenia and 1.66 times higher in women with sarcopenia.

There were already many research on the impacts of sarcopenia in elderly patients, including those who underwent surgery and orthopedic surgery. Xia et al. (2020) conducted a review meta-analysis of 54 observational studies with a total of 1,851 participants. Among these 54 studies, 21 studies were about the survival outcome of patients with cancer. As shown in 20 (95%) out of the 21 studies, patients who had sarcopenia also had worse survival outcomes than those who did not. The sarcopenic patients with gastric cancer have the most affected survival outcomes. Other ten age-related studies showed that sarcopenic patients >65 years old had 1.5 times higher rate of hospitalization, almost 2 times higher chance of fracture, and a higher risk of falls and readmission. The risk of falls in elderly patients leads to a higher risk of fragility fractures. A cohort study by Laubscher et al. (2020) presented that 34 (52%) out of 65 South African patients, who underwent hip surgeries of fragility fractures in the hip that were caused by low-velocity trauma (mechanical forces that should not result in fracture), had sarcopenia. Yeung et al. (2019) meta-analyzed 33 studies on the impacts of sarcopenia that include higher risk of falls and fractures. There were up to 1.6 times higher risk of falls and 1.84 times higher risk of fractures in elderly aged >65 years with sarcopenia.

A cross-sectional study by Iijima & Aoyama (2021) researched the increased risk of falls experienced by older adults with knee osteoarthritis (KOA) and sarcopenia. There were 291 participants, with more than three quarters being women in the age range of 60-90 years old. The results showed that the sarcopenic participants with KOA had an increased prevalence of single and multiple falls than those without KOA. Also, the sarcopenic participants with KOA had an increased risk of recurrent falls up to four times higher than those without the conditions.

Another prognostic factor of sarcopenia is mortality. Sarcopenia is an independent mortality risk (Arango-Lopera et al. 2013). A study by Deren et al. (2017) analyzed the one-year mortality of 99 participants aged ≥60 years who had sarcopenia and acetabular fractures. One-year mortality was more prevalent in sarcopenic patients with acetabular fractures than patients without those conditions. Demographically, those with sarcopenia also had a far lower mean BMI (23.6 kg/m2) than the non-sarcopenic group (31.7 kg/m2). However, there were some complications that was less prevalent in the sarcopenic group, such as complications of urinary tract infection that was more common in the non-sarcopenic group.

Muscle mass, as one of the criteria to establish the diagnosis of sarcopenia, plays a significant role in orthopedic patients. It has been known that declining muscle mass is positively correlated with aging. As people get older, they tend to be less mobile than the younger group. The decline in muscle size/mass can limit their movement, especially if they have hip fractures. Men and women aged 18 to >65 years have respectively 0.47% and 0.37% median values of muscle mass loss per year. If they are >75 years old, then the rate rises to 0.64%-0.70% for women and 0.8%-0.98% for men per year.

Not only muscle mass, muscle strength also declines as people age, especially in elderly aged >75 years, with up to 4% for men and 3% women per year (Mitchell et al. 2012, Chen et al. 2021). An observational study by Chen et al. (2021) analyzed the declining muscle mass and function with a prognosis of sarcopenia using the AWGS criteria one year after geriatric hip fracture surgery. Participants with sarcopenia had a tremendous loss of upper and lower limb muscle mass (ASM) and lower limb skeletal muscle mass (LSM) compared to the non-sarcopenic participants. The mean loss of ASM in the sarcopenic participants was 9.18%, compared to 1.15% in the non-sarcopenic participants. The mean loss of ASM in the sarcopenic participants was 9.18%, compared to 1.15% in the non-sarcopenic participants. Whereas, the mean loss of LSM was 10.27% versus 2.48% in sarcopenic and non-sarcopenic participants, respectively.

As previously described, people tend to be inactive and move less as they get older. It is aggravated by degenerative diseases, such as degenerative lumbar spinal stenosis (DLSS). This disease causes a long-term back pain and/or leg pain in elderly patients, which results in inactivity. In a prospective case-control study by Park et al. (2016), patients with sarcopenia had a higher prevalence of DLSS than patients without such condition. Sarcopenic patients with DLSS also had worse TUG test (physical performance test) than those without sarcopenia and DLSS.

Many studies have concluded that there is a deleterious effect of sarcopenia on musculoskeletal pathology. However, one study on upper extremity pathology and rotator cuff tears by Atala et al. (2021) showed that there was no significant difference between those with and without sarcopenia in the prevalence of rotator cuff tears. The result of the study is also supported by another study by Han et al. (2021), who showed that

sarcopenia was not associated with an increased risk of rotator cuff tendon tears, although the prevalence of shoulder pain was higher in the sarcopenic group than the non-sarcopenic patients. Han et al. (2021) used MRI, while Atala et al. (2021) used ultrasound, to evaluate the rotator cuff tendon tears.

As stated in the 2019 updated AWGS consensus, the goal of sarcopenia treatment is still uncertain, whether to change the sarcopenia status to be non-sarcopenic, or improve the patients' muscle mass, strength, and physical performance (Chen et al. 2020). Nevertheless, exercise and nutrition are the mainstay of treatment to improve the individual components of the sarcopenia criteria.

The current recommendation of exercise to counteract sarcopenia for adults is resistance exercise (RE) or weight/strength training. RE is a type of exercise that uses muscles to hold and/or contract against weight. The weight can be from one's own bodyweight, resistance machines, free weights, and resistance bands. RE can increase muscle strength, mass, and physical performance. It is recommended as the first-line treatment for sarcopenia (Hurst et al. 2022).

The main principles of RE are specificity, overload, and progression. The aim of these three principles is to improve physical ability or skill. Thus, training stimulus (acute or chronic response to training, such as physiological stress caused by RE) should be in accordance with these principles. In a literature review by Hurst et al. (2022), specificity means that the training responses are targeted to a specific muscle group, so that the capability of doing specific action will improve, e.g. training the muscle group of lower body will eventually help improve the capability of rising from a chair. The second principle, overload, means that the heavier the weight load/resistance a patient is able to overcome, the more adaptability will be produced. Thus, the weight must be increased gradually. Lastly, progression means that more frequent increase of weight is needed as the body becomes adaptable. The frequency of RE should be two sessions per week, with more training on the lower body muscle group than the upper body muscle group because the lower body muscle group is the basis performing daily activities (e.g. walking, climbing stairs, and rising from a chair).

A randomized controlled trial (RCT) by Otsuka et al. (2022) showed that moderate-intensity RE improved the quality and quantity of lower muscle group, such as quadriceps and vastus lateralis muscle, in men and women aged 50-79 years compared with those who did not exercise in the 24 weeks trial. Another study to support the positive effect of exercise on sarcopenia is presented by Shen et al. (2022) who concluded that there was an improvement of grip strength, knee extension strength, walking speed, and faster TUG test in sarcopenic patients that implemented exercise intervention. Although RE is considered the first-line treatment of sarcopenia, lower intense exercise also has positive impacts on sarcopenic patients. A retrospective cohort study by Yoshimura et al. (2022) analyzed 302 stroke patients with sarcopenia who underwent rehabilitation programs including low-intensity exercise. The program used chair-stand exercise, in which patients were asked to perform 120 times sit and stand task for 20 minutes. At discharge, the prevalence of sarcopenia in the patients who underwent the program declined significantly (21.9%) from 100% to 78.1%.

Strength and limitation

The strength in this study is the importance of diagnosing and treating sarcopenia. The importance of exercise and adeuate nutrition intake as the main treatments for sarcopenia, with resistance or strength training being recommended as a firstline treatment. The knowledge of sarcopenia is essential for clinicians and surgeons in anticipating the implications of the condition and taking appropriate action to treat it.

CONCLUSION

Sarcopenia is a heavy burden on the elderly around the world, as almost one in ten of them are affected. This heavy burden must be prevented. In order to mitigate this problem, patients and doctors need to widen the knowledge of sarcopenia. Now the problem of the utmost importance for clinicians and surgeons is to realize that this comorbidity can cause deleterious effects on patients, such as complications, falls, fractures, morbidity, mortality, longer hospitalization, financial problems, and even readmission to the hospital. Although sarcopenia presents the risk of those complications, this comorbidity can be overcome by doing resistance exercise regularly and fulfilling adequate nutrition intake. Therefore, knowledge of this comorbidity should be a trigger for clinicians and surgeons to evaluate the treatment plan of sarcopenia and also act better to anticipate the implications of sarcopenia experienced by the patients.

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Conflict of interest

The author reports no conflict of interest in this work.

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Author contribution

AF, DI, and ARBA contributed to the design of the research and data analysis. All authors contributed equally in conducting the study and also writing and revising the manuscript.

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Systematic Review

A MODEL OF PERFORMANCE EVALUATION FOR HEALTHCARE WORKERS BASED ON SATISFACTION AND REMUNERATION (FINANCIAL AND NON-FINANCIAL)

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ABSTRACT

*This study aimed to formulate an evaluation model of the performance of health workers from the perspective of financial and non-financial satisfaction and remuneration. The research method used was a literature study with a literature review approach. The research findings were the healthcare workers' performance evaluation model based on satisfaction and remuneration. The evaluation was conducted by assessing the employee performance targets (*sasaran kerja pegawai*/SKP) and employee satisfaction related to the financial and non-financial remuneration, Job Description Index (JDI), competencies, and motivation. In addition, this model has been also used for the evaluation of performance appraisal based on remuneration among employees (i.e. health workers) in government agencies, especially hospitals.*

Keywords: *Remuneration; hospital; Job Description Index; health system; health policy*

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Highlights:

1. This study aimed to formulate an evaluation model of the performance of health workers.
2. Employee performance targets and employee satisfaction were assessed by considering the financial and non-financial remuneration, Job Description Index (JDI), competencies, and motivation.
3. The performance evaluation model for health workers shows the importance of remuneration for government agencies, employees, and employee performance.

INTRODUCTION

The three-year impact of the COVID-19 pandemic has hit the world. It does not only raising health and economic problems, but also problems in managing institutions or organizations (Arrasyid et al. 2019, Prakoeswa et al. 2021). A survey for the last three years (2019-2021) reported the satisfaction with the health system and health policy among the employees of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The report stated that the employees were wholly or partially dissatisfied (Figure 1). If observed in more detail, the dissatisfaction occured because the components of income and compensation received were perceived to be unsatisfactory. This phenomenon is interesting to explore because the compensation budget for Dr. Soetomo Hospital employee pay tends to increase from year to year. More than 30% of revenue is allocated for employee remuneration.

Research on performance evaluation based on employee satisfaction showed that compensation (remuneration) will not directly lead to employee satisfaction. Although previous research have shown no direct relationship between pay and performance, this perspective can potentially cause problems (problem-prone) and, of course, will hinder the achievement of vision and mission in hospitals. Other research showed that compensation allegedly affects motivation (Sancoko 2011, Pratama & Prasetya 2017, Rahayu & Ruhamak 2017, Rahayuningsih et al. 2019). This concern is supported by a research which found that the remuneration system impacts the quality of employee performance, increases productivity and loyalty, prioritizes customer satisfaction, and avoids corruption (Calvin 2017).

Nasution et al. (2021) found that an employee's potential is not seen in quantity but in quality. Competencies, skills, development, creativity, initiative, problem solving, predicting, thinking proactively, and adaptability are the several aspects that influence employee performance. Thus, if problems regarding compensation can be resolved, then the risk of problems in employee performance will be reduced.

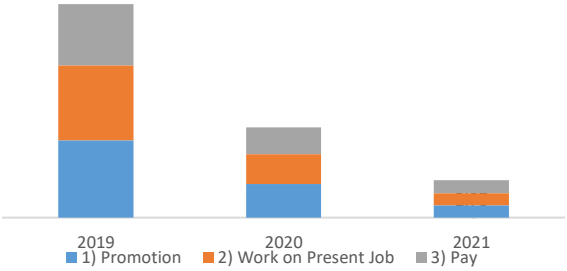

Figure 1. Percentage of three indicators of JDI at Dr. Soetomo Hospital (2019-2021).

Based on the background that has been described, employee performance is influenced by satisfaction and compensation. However, there has been no research on the formulation of a model for evaluating the performance of healthcare workers based on satisfaction and compensation (financial and non-financial) at Dr. Soetomo Hospital. Therefore, this study aimed to formulate a model for evaluating the performance of health workers based on satisfaction and compensation (financial and non-financial).

MATERIALS AND METHODS

This study was a qualitative research using a literature study approach. The five stages of the research procedure used were: 1) planning and performing the search strategy; 2) carrying out initial screening by removing duplicates, determining the inter-rater reliability, and examining the articles per the inclusion and exclusion criteria; 3) data extraction; and 4) data syntheses. The data that had been verified were then analyzed, and the structure of the components of each aspect was constructed to produce a model according to the research objectives.

RESULTS

Based on the 2021 report from the staffing database of Dr. Soetomo Hospital, the number of employees were 4,223. The employees were grouped by professions, which consisted of 593 general practitioners and specialists, 1,486 nursing staffs, 697 other health workers, and 1,486 non-health workers. The number of employees of Dr. Soetomo Hospital was the largest compared to other hospitals in Indonesia.

Employee remuneration can be in the form of salary, fixed allowance, variable allowance, and incentives. Compensation can be interpreted as the take-home pay obtained by employees as a reward for the work that has been carried out. The remuneration components are salary, incentives, and bonuses. Compensation is paid periodically, weekly, monthly, or yearly to the workers. Incentives can be interpreted as additional income specific to each employee depending on the results of the employee's assessment. At the same time, bonuses can be construed as extra income given to employees when targets have been exceeded.

Satisfaction is a person's feelings when they get what they expected (Haedar et al. 2016). Job satisfaction differs from one indvidual to another (Potale & Uhing 2015). In addition to the customer satisfaction index, the employee satisfaction index also includes outcome parameters. Measuring the satisfaction index of hospital employees enable the hospital management to be informed whether they are doing well and can be used as a medium for establishing effective communication. The employee satisfaction index is also used as a training needs assessment (TNA) and to facilitate the recruitment of new and retired employees (Palagia et al. 2012).

The employee satisfaction index can determine how loyal employees are to an organization. Several indicators can be used to measure employee satisfaction with the workplace. One of the indicators is the Job Description Index. It is an indicator used to assess employee satisfaction which focuses on five components, including general job description/the job itself, rewards received/pay, job promotion opportunities/promotion, assessment of subordinates/ supervision, and closest colleagues/coworkers (Smith et al. in Agustia 2018).

Performance

Several factors and conditions that determine the employee performance in a company either come from the internal or external factors of the employees. Mangkuprawira & Hubeis (2007) stated that performance is the result of a work carried out by a person for the organization at a specific time. Another definition of employee performance comes from Murphy and Cleveland, as cited in Pasolong (2007), who stated that performance is the quality of tasks and work of an employee. Performance is about doing a job and the results obtained from that job (Baron & Armstrong 2007). Simanjuntak (2005) defined performance as the degree of achievement that is resulted from carrying out specific tasks.

An employee's performance can be influenced by various factors that can be classified into three groups: personal competence, support from the organization, and support from the management. From the research that have been found, it can be concluded that performance is the result of a worker's actions in accordance with their work and supervised by certain people, i.e. supervisors or leaders of the organization.

Performance assessment in government environment

Gomes & Saraiva (2001) found that performance appraisal or evaluation is a way to measure the contribution of individual members of an organization. Performance appraisal or evaluation is not only needed to determine individual contributions, but also corporate contributions. Bernardin and Russell, as quoted by Gomes (2003), explained that there are eight indicators of employee performance: 1) quality of work, which shows how the work are carried out based on the abilities and requirements of an individual employee; 2) quantity of work, the amount of work done in a certain period; 3) knowledge of work, level of knowledge and skills in the field of work; 4) creativity, originality of ideas that arise from actions to solve problems; 5) cooperation, willingness to work together and communicate with other people (fellow members of the organization); 6) reliability, being trustworthy regarding the presence and completion of work; 7) initiative, always enthusiastic in facing new tasks and expanding their responsibilities; and 8) personal quality, which relates to leadership, friendliness, and integrity of the individual employee.

Based on the government regulation of the Republic of Indonesia number 30 of 2019, which regulates the performance assessment of civil servants, and the regulation of the Minister of Administrative and Bureaucratic Reform number 6 of 2022 regarding the performance management of civil servants, performance appraisals aim to ensure that coaching is carried out objectively and based on achievement and career paths (Republic of Indonesia 2019, Minister of Administrative and Bureaucratic Reform 2022). The basis for the assessment is the organizational level performance planning which is suited to the individual level of employees by taking into account the targets to be achieved, the realization of achievements, and the components of employee behavior.

Employee performance targets (*sasaran kerja pegawai*/SKP) oversee the performance that is expected to be achieved annually by each individual. An evaluation of employee performance at least includes the individual performance indicators (*indikator kerja individu*/IKI) and their targets. Individual performance indicators consider the following components: a) specific, must be specific to the individual employee, not all employees in general; b) measurable; c) realistic, realistically achievable; d) time-bound, meaning that it has a deadline for achievement; and e) adjusted to internal and external conditions of the organization. The targets of employee performance consist of four components, i.e. quantity, quality, speed of completion of work, and cost. Work behavior indicators consist of five components, i.e. service orientation, commitment, work initiative, cooperation, and leadership (Republic of Indonesia 2019, Minister of Administrative and Bureaucratic Reform 2022).

Job satisfaction theories

Robbins & Judge (2011) defined job satisfaction as a positive feeling about work by considering the various components of the job. Job satisfaction can contribute to life satisfaction. The nature of the environment around an individual outside of work will also influence their feelings towards their work. Similarly, because work is an essential part of life, job satisfaction affects an individual's overall life satisfaction (Davis & Newstrom 2000). There are various interpretations of job satisfaction. Davis & Newstrom (2000) interpreted job satisfaction as one of the motivating factors for employees to appreciate and improve the quality of their work, so that the organization can obtain positive results from the employee performance.

There are three theories that are closely related to job satisfaction, i.e. the expectancy theory, two-factor theory, and theory of justice. The expectancy theory was proposed by Victor Vroom who stated that someone executes a task from work with a hope that they can achieve the expected results. These expectations are addressed to the organization as the organizer of the work. Usually employees will expect a reward for working (Fauziana et al. 2021, Diwyarthi et al. 2022).

The two-factor theory states that there are two type of factors that will have an influence on individuals in carrying out work activities. The first one is the motivational factor that triggers satisfaction, while the second one is the hygiene factor that triggers dissatisfaction. The motivational factors are closely related to human psychology, such as getting recognition and feeling happy when work has been completed. On the other hand, dissatisfaction can occur when the hygiene factors, e.g. the provision of remuneration, are not met (Tama & Hardiningtyas 2017, Lantara & Nusran 2019).

The theory of justice explains what is felt by individuals regarding how they are treated in the context of doing work. They will feel they are being treated fairly if they are benefiting from their work, but if they are harming themselves, then they will feel that they have been treated unfairly (Bangun 2012). Based on these three theories, it can be concluded that job satisfaction in the context of a hospital is a feeling conveyed by health workers through their attitude and behavior toward their work conditions.

Dipboye et al. (1994) stated that the Job Description Index is the most widely used measure of job satisfaction in research because it provides a reliable and valid job satisfaction scale. Smith et al. (in Agustia 2018) described job satisfaction as a feeling or a response to the components of work conditions. There are five dimensions of job satisfaction based on that definition: 1) the job itself*:* this dimension measures what employees feel about the work they are currently doing. It also measures authority, variety of tasks, opportunities to increase knowledge, responsibilities given, and the amount of works; 2) pay: this dimension measures how employees feel about the difference between the rewards they expected and received; 3) promotion opportunity: this dimension measures what employees feel in terms of promotion administration in an organization that includes the opportunities to get promotions or develop their careers; 4) supervision*:* this dimension measures an employee's satisfaction with their leader, whether they are able to provide assistance, as well as provide technical guidance on what the employee is doing; and 5) coworkers: this dimension observes the relationship and satisfaction experienced by the employee about their coworkers, e.g. how kind and caring employees are to one another.

Principles and components of remuneration

Remuneration comes from the word "remunerate" which means "to pay". Remuneration will be given to employees because there is an employment relationship between employers and employees where remuneration can be given once a job has been completed. There are many similarities between remuneration and compensation. The difference is that compensation, in addition to financial rewards, also includes non-financial rewards, while remuneration focuses on financial rewards (Roberia 2009). The remuneration system has several elements, including those who give and those who receive, job grade basis, a calculation formula, how to give it, and a time limit for provision along with the consequences if there is a violation. The implementation of remuneration in an organization must be regulated in guidelines and must meet several principles, including the principles of decency, equality, proportionality, and performance.

Remuneration has become an obligation and an inseparable tool of government and private organizations. Most organizations have already implemented a remuneration system. One of the reasons is that there are many regulations from the government regarding the remuneration system, e.g. government regulations for organizations with the status of public service agencies (*badan layanan umum*/BLU). Management officials, supervisory boards, and employees of BLU may be given remuneration according to government regulation number 23 of 2005 concerning financial management of public service agencies (Republic of Indonesia 2005). The regulation includes hospitals where most of the employees are health workers who are the recipients of remuneration.

Sopiah et al. (2020) described remuneration as one of the several factors that affect employee performance in carrying out their main duties and functions. Remuneration is given by an organization to its employees in order to achieve organizational goals. With the provision of remuneration, employees are required to always optimize their work and achieve certain targets through their performance. It can be summarized that remuneration is a system that regulates the provision of compensation based on job grade and calculation formula. It is assessed by considering the performance achievements and responsibilities which are accumulated into points. The provision of remuneration must take the principle of proportionality, equality, propriety, and time into account.

Santoso & Riyardi (2012) explained the five principles of remuneration provision, including: 1) decent and reasonable: remuneration must be equally beneficial to the provider (organization) and the recipient (employee); 2) merit system: the basis of remuneration is the routine evaluation of employee performance appraisal; 3) competitive in nature: remuneration must be competitive to attract employees who have unique competencies to carry out their main duties and functions as well as possible; 4) transparent: employees must be able to understand and obtain information on how the organization determines salaries, benefits, and the terms of the increases; and 5) fair: remuneration does not have to be the same amount for each employee, but must take into account several factors that are mutually beneficial and just for the provider and recipient.

Minister of Health of the Republic of Indonesia (2014) issued the regulation number 68 of 2014 concerning guidelines for the preparation of the remuneration system. There are three components of the remuneration system, i.e.: 1) pay for position: the amount of pay received by employees is not affected by the hostpital (employer) income because it is based on rank, position, length of work, and education level, which includes allowances (family allowances, professional allowances, job allowances); 2) pay for performance: the amount of pay received by employees is closely related to hospital income and based on the employees' type of work, workload, and work achievement which are scored according to the leadership and policy of the hospital (employer); 3) pay

for people: the amount of pay is highly dependent on the conditions and requirements determined by the hospital (employer) and given if the organization achieves the performance goal. The pay for people is usually known as bonus or appreciation, for example in the form of official travel expenses, insurance premiums, recreation costs, Umrah or Hajj, pension insurance, etc.

DISCUSSION

Human resource management is the most important component of hospital management. This is because hospitals are labor-intensive organizations. The types of staff in hospitals are very diverse. They are expected to have higher work motivation, integrity, and loyalty, but those are not the same among individuals, which will ultimately affect their job satisfaction.

Remuneration is a compensation or wage given to employees because of their contribution to the organization by performing their best. Remuneration is an important aspect for employees and the organization itself. Literatures have shown that remuneration can lead to motivation and encouragement in employees so that they always work more diligently. In addition, remuneration can play a role in creating high performance. The provision of remuneration can be an effective step to spur employees to perform well because their performance will have a direct impact on the amount of rewards received. In other words, remuneration is used as a bond that the organization uses on its employees and is capable of attracting prospective employees.

Job satisfaction is something that is felt by an individual employee regarding their work. In other words, it is a form of emotional attitude from the employees whether they fancy their jobs or not. Job satisfaction is something that needs attention because human resources are the most important factor for an organization to succeed in achieving its goals.

Job Description Index (JDI) is a measuring tool that is often used to measure job satisfaction. There are five important components in job satisfaction, including work in general (the job itself), rewards (pay), superiors (supervision), promotion opportunities (promotion), and coworkers.

Performance shows the level of achievement of an individual employee at a certain time in carrying out their duties and functions. Employee performance is evaluated by comparing it to the standard of the work and targets to be achieved, which were originally planned and agreed upon together. Performance improvement will not be achieved if the organization has not implemented human resource management effectively, which enable the individuals to be competitive in improving their performance. The Cascade approach proposed that the strategies used to achieve organizational goals should be translated to the individual level. The informations obtained from the performance appraisal enable the leader to observe employee achievements and consider them when making decisions or taking necessary actions.

Employee performance targets (*sasaran kerja pegawai*/SKP) are plans and targets that should be achieved through employee performance. The plans and targets are made by the employees themselves, then must be achieved within a certain period. Performance targets must be determined, known, and approved by the individual's direct supervisor in respect to their tasks and responsibilities. Human resources management theory explains that remuneration can have an influence on employee satisfaction, motivation at work, and performance.

Non-financial compensation is similar to remuneration, but does not involve money. One of the characteristics of non-financial compensation is the satisfaction gained from performing meaningful work-related tasks, Some of the examples are training to improve skills and programs to create pleasant working conditions and environments, such as provision of tourism programs, canteens, places of worship, and sports facilities.

Motivation is generally known to have a positive influence on the employee performance although it is not the sole factor that can shape performance. Employees are motivated when they get attention and praise from the leadership. If the employee motivation is adequate, the performance will also increase.

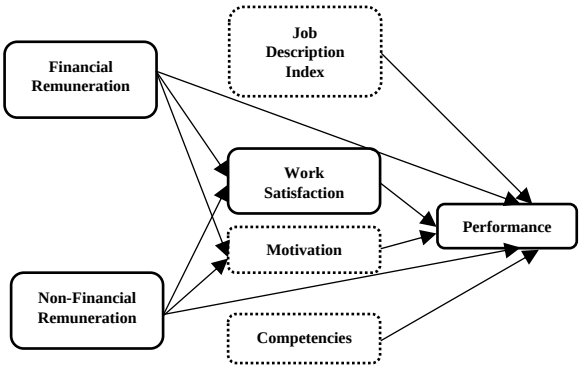

Figure 2. Performance evaluation based on satisfaction and remuneration

Job satisfaction is a positive feeling about a job. It is also a driving factor for increasing employee performance, so that it will contribute in improving

organizational performance. From the discussion, the model of employee performance evaluation based on satisfaction and remuneration (compensation), especially for health workers, can be formulated.

Strength and limitation

The model has been used for the evaluation of performance appraisal based on remuneration among health workers in government agencies, particularly hospitals, adding to the strength of the research's relevance and potential impact. Taken together, the sentence effectively conveys the aims, methods, and findings of the study, highlighting the importance of considering financial and non-financial factors in evaluating the performance of healthcare workers.

CONCLUSION

The performance evaluation model for health workers in this study shows the importance of remuneration for government agencies (especially hospitals), employees (especially health workers), and employee performance. This model is based on the employee performance targets (*sasaran kinerja pegawai*/SKP) and employee satisfaction that is measured through the financial and non-financial remuneration, Job Description Index (JDI), competencies, and motivation.

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Conflict of interest

The authors report there was no conflict of interest.

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Author contribution

J.W. conceived the presented idea and developed the theoretical framework of the literature review. N.H. and Q.A. checked the sources, verified the analytical methods, and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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Systematic Review

CASTOR PLANT (*Ricinus communis* L.) LEAF EXTRACT AS POTENTIAL ANTIBACTERIAL AGAINST THE GROWTH OF *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis *is the cause of pulmonary tuberculosis that can reduce human health. In the therapy of the disease, patients can develop resistance to tuberculosis drugs. Based on the 2015 health profiles of Indonesia, 15,380 people were suspected to have multidrug-resistant tuberculosis (MDR-TB), while 1,860 people were confirmed patients with MDR-TB. There is a need for innovation to develop the latest treatments using natural ingredients, one of which is castor plant (*Ricinus communis *L.) that contains antibacterial compounds against* Mycobacterium tuberculosis*. This study aimed to understand the antimicrobial potential of castor plant (*Ricinus communis *L.) leaf extract against the growth of* Mycobacterium tuberculosis*. This scientific paper was a quantitative systematic review study. Literature in the form of journal articles and books were obtained through search engines, i.e. ebook database, Google Scholar, Cochrane, Wiley, and PubMed. The results of the literature source search were 19 journal articles and 4 ebooks, as well as 4 journal articles that were in accordance with the title of this literature review and discussed the effects of castor plants on the growth of* Mycobacterium tuberculosis*. The results of the analysis showed that castor plant (*Ricinus communis *L.) leaf extract has the potential in the antibacterial activity against the growth of* Mycobacterium tuberculosis *because it contains phytochemicals in the form of flavonoids, saponins, alkaloids, tannins, and fatty acid amides derived from ricinoleic acid as the main constituent of castor plants (*Ricinus communis *L.). There is antimicrobial potential for castor plant (*Ricinus communis *L.) leaf extract against the growth of* Mycobacterium tuberculosis.

Keywords: *Castor plant leaf; minimal inhibitory levels;* Ricinus communis *L.; lung health,* Mycobacterium tuberculosis*; tuberculosis*

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Highlights:

1. Patients experiencing failure of first-line drug and developing multidrug resistant tuberculosis (MDR-TB) has increased throughout 2011-2015.
2. Castor plant leaf extract (*Ricinus communis* L.) has an antibacterial potential against the growth of *Mycobacterium tuberculosis* bacteria.

INTRODUCTION

Mycobacterium tuberculosis is one of the most dangerous Gram-positive pathogens that cause tuberculosis (TB) (Aljanaby et al. 2022). It is one of the deadliest bacteria and the main causative agent of TB, one of the most dangerous infections that has killed thousands of people worldwide because the bacteria grow slowly and early diagnosis for prevention is difficult (Rabahi et al. 2017, Ekawati 2018, Gopalaswamy et al. 2020). Statistics showed that one-third of the world population has underlying TB infection (Houben & Dodd 2016). Globally, more than a million people die each year as a result of the active disease, making it the second leading cause of death worldwide after the human immunodeficiency virus (MacNeil et al. 2020). According to the 2015 report of the World Health Organization (2015), the estimation of new TB cases was a million per year (399 per 100,000 population), with 100,000 deaths per year (41 per 100,000 population). The estimated number of human immunodeficiency virus (HIV)-positive TB was 63,000 cases (25 per 100,000 population). The Case Notification Rate (CNR) of all reported cases was 129 per 100,000 inhabitants. The total number of cases was 324,539, of which 314,965 were new cases (Indonesian

Ministry of Health 2016). In 2017, the number of new TB cases in Indonesia was 420,994. Based on gender, the number of new TB cases in 2017 was 1.4 times greater in men than in women. A prevalence survey, with a ratio of men and women, revealed that men are three times more at risk of TB compared to women because they are more exposed to TB risk factors, such as smoking and non-compliance of medication. The survey found that 68.5% of the male participants smoked, while only 3.7% of the female participants smoked (Indonesian Ministry of Health 2018).

The main symptoms of TB patients are hunger, fever, weight loss, drenching night sweats, feeling very tired or lacking energy, cough, and weight loss. Routine laboratory tests are rarely helpful in diagnosing TB (Lyon & Rossman 2017, Usmani et al. 2018). The nature of the disease is progressive chronic and its treatment takes as long as six months, with drug administration in the initial two months using isoniazid, rifampicin, and pyrazinamide. In the next four months, the treatment continued with drug administration using isoniazid and rifampicin (Rabahi et al. 2017). In the recovery of patient after receiving therapy for six months, some experience therapeutic failure or TB drug resistance, including mono-resistance, polyresistance, multi-drug resistance (MDR), extensively drug resistance (XDR), and rifampicin resistance (Velayati & Farnia 2016, Manson et al. 2017, Esteban & García-Coca 2018). In multidrug-resistant tuberculosis (MDR-TB) cases recorded in 2015, there were 15,380 suspects and 1,860 confirmed patients (Indonesian Ministry of Health 2016).

Medicinal plants offer great hope of meeting the needs of treatment and have been used for centuries to treat a wide variety of ailments. Recently, several reports and reviews of medicinal plants and natural products with anti-mycobacterial activity have been published. Medicinal plants contain compounds that may act as natural antitubercular agents in tubercular activity, such as alkaloids, peptides, tannins, phenols, quinones, and triterpenoids (Copp 2003, Okunade et al. 2004, Arya 2011, Rashid et al. 2015). Sixty plant species were studied for TB treatment, and 90 for leprosy treatment. In the Ayurvedic system, they have potential against tuberculosis, leprosy, and related disorders (Collins & Franzblau 1997).

The treatment of TB disease using medicinal plants is required. There is a need for innovation to develop the latest treatments using castor plants (*Ricinus communis* L.). Research on the utilization of castor plants are still few and rarely done. The use of castor plant leaves offered a potential of antimicrobial (Suvarna et al. 2018, Ghramh et al. 2019). Phytochemical analysis on castor plant (*Ricinus communis* L.) leaves revealed compounds that include tannins, saponins, alkaloids, and flavonoids, which are the basic properties of medicinal plants and the primary ingredients for the production of new drugs (Kumar 2017, Suurbaar et al. 2017, Azmy 2020). In previous studies, *Ricinus communis* L. leaf extracts were dissolved in water and partitioned using n-hexane to produce soluble n-hexane, then tested on *Mycobacterium tuberculosis* H37Rv (sensitive to all first-line drugs) and obtained a minimum inhibitory level (MIL) of 10 mg/mL (Ullah et al. 2017).

MATERIALS AND METHODS

The data search used a database of ebooks, Google Scholar, Cochrane, Wiley, and PubMed with the keywords "*Ricinus communis* L. + anti microbe", "*Ricinus communis* L. + anti-bacterial", "*Ricinus communis* L. + *Mycobacterium tuberculosis*", "extract leaf *Ricinus communis* L. + *Mycobacterium tuberculosis*", "castor + *Mycobacterium tuberculosis*", "extract leaf castor + *Mycobacterium tuberculosis*", "*Mycobacterium tuberculosis*" to collect literature from 2000 to 2021 for systematic review. The criteria for journal articles collected were as follows: 1) full text journal articles, 2) research on castor (*Ricinus communis* L.) as an antimicrobial and anti-*Mycobacterium tuberculosis*, 3) contain information about castor plant (*Ricinus communis* L.), 4) contain information about *Mycobacterium tuberculosis*, 5) literature in the form of qualitative, quantitative, and mixed method studies, 6) articles from quartile 1-4 journals.

The literature search resulted in 19 journal articles and 4 ebooks. Among journal articles that were in accordance with the title of this literature review, 4 articles discussed the effects of castor plants on the growth of *Mycobacterium tuberculosis*, while 9 articles discussed taxonomy, phytochemical content, and morphology in castor (*Ricinus communis* L.). There were 5 journals and 4 ebooks that discussed taxonomy, morphology, culture methods, and identification methods of *Mycobacterium tuberculosis*. There was also an article that discussed the effect of castor (*Ricinus communis* L.) leaf extract on minimum inhibitory levels of *S. aureus, B. subtilis, P. aeruginosa,* and *K. pneumoniae*.

RESULTS

Mycobacterium tuberculosis is a species of pathogenic bacteria in the Mycobacteriaceae family (Gupta et al. 2018). It is the causative agent of tuberculosis with a high mortality rate in Indonesia. Data recorded from 2011 to 2015 showed that there were increases of MDR-TB suspects from 1,255 in 2011 to 2,441 in 2012, 3,833 in 2013, 9,399 in 2014, and 15,380 in 2015. Whereas, confirmed patients with MDR-TB were 216 in 2011, 460 in 2012, 1094 in 2013, 1,752 in 2014, and 1,860 in 2015. It indicated that there was a high increase of MDR-TB cases from 2011 to 2015 in patients who failed first-line drug therapy and caused them to develop MDR-TB (Pfyffer 2015, Indonesian Ministry of Health 2016).

The growth of *Mycobacterium tuberculosis* in adults is a slowly progressive process characterized by chronic inflammation, caseation, and formation of cavities. The central nuclei can rupture into the bronchi, allowing large numbers of organisms to spread to other areas of the lungs and become aerosolized with coughing, thereby infecting others (Barbier & Wirth 2016). Mycobacteria are aerobic, although some species can grow under a reduced O_2 atmosphere. The high complex lipid content of the cell wall prevents access to common aniline dyes. Although not directly stained by the Gram method, mycobacteria are usually considered Gram-positive. When stained by special procedures (e.g. Ziehl-Neelsen stain), mycobacteria were not easily decolorized, even with acid-alcohol. Compared to other bacteria, the growth of most mycobacterial species was slow, up to 20 hours on commonly used media. A natural division existed between slow-growing and fast-growing mycobacterial species (Drapal & Fraser 2019). Slow-growing mycobacteria required more than seven days to colonize on solid media from aqueous inoculum under ideal culture conditions. Fast-growing mycobacteria took less than seven days when subcultured on Löwenstein-Jensen (LJ) medium, but took several weeks to emerge in primary culture from clinical specimens (Pfyffer 2015). The identification of *Mycobacterium tuberculosis* bacteria was conducted by taking pulmonary secretion specimens from the bronchial tree. This specimen was obtained from spontaneous sputum, induced sputum, gastric lavage (for children or seriously ill patients who did not produce sputum), bronchoalveolar lavage (BAL), and transtracheal aspiration (endotracheal tube) (Mertaniasih 2019). The identification of *Mycobacterium tuberculosis* bacteria was also performed through microscopic examination of acid resistant rods, which included the standard Ziehl-Neelsen staining of sputum smear on standard binocular microscopes and fluorescein staining of sputum smear using microscopic fluorescents or LEDs (e.g. acridine orange) (Mertaniasih 2019).

Active plant extracts were tested using broth microdilution technique. The Middlebrook (MB) 7H9 broth was prepared and sterilized according to the manufacturer's instructions. A fresh working extract solution was prepared by diluting the stock solution to a concentration ranging from 2.5 to 40 mg/mL and adding 5 L of mycobacterial sensitive strain, the inoculum H37Rv (104-105 CFU/mL). Experiments were carried out after incubation at 37°C for four weeks (Ullah et al. 2017). In a previous study, the leaf extract of *Ricinus communis* L. was dissolved in water and partitioned using n‑hexane to produce soluble n‑hexane and then tested on *Mycobacterium tuberculosis* H37RV (sensitive to all first-line drugs) (Ullah et al. 2017).

Table 1. Average number of colonies of *Mycobacterium tuberculosis* H37RV on *Ricinus communis* L. leaf extracts

	Method	Solvent	Concentrate	Colony Total Average
Ladda & Magdum (2012)	Dilution	Ethanol	100 µg/mL	20.33
			150 µg/mL	4.33
			200 µg/mL	0
		Acetone	100 µg/mL	17.33
			150 µg/mL	0
			200 µg/mL	0
		Chloroform	100 µg/mL	55.66
			150 µg/mL	36
			200 µg/mL	18.33

The fatty acid amides derived from ricinoleic acid were tested. Ricinoleic acid (C18:1, OH) or 12-hydroxy-9-cis-octadecenoic acid is a major constituent (80-90%) in the hydroxyl chain of castor (*Ricinus communis* L.) oil. The fatty acid amides derived from ricinoleic acid showed interesting results against tuberculosis strains. The compound ricinoleylpyrrolidilamide, (R, R)-12d, showed antitubercular activity, with a minimum inhibitory level of 12.5 g/mL for tension resistance (D'Oca et al. 2010). The results were expressed as the ratio of resistance compared to control cultures. Fourteen colonies of *Mycobacterium tuberculosis* H37RV were observed for the ethanolic extract of *Ricinus communis* L. at 100 g/mL, while ten colony counts were observed for the patient strain at 150 g/mL.

Methanol inhibited the growth of *Mycobacterium tuberculosis*, which interpreted as nitrate reductase assay (NRA), while acetone extract did not inhibit the growth of *Mycobacterium tuberculosis*. The methanol had antitubercular activity at 100 g/mL (Ladda et al. 2018). The MIC for the standard strain and patient strain was 100 g/mL and 150 g/mL respectively. The resistance ratio (RR) was found to be 1.5. Because the RR value was less than 2, it indicated that *Mycobacterium tuberculosis* was sensitive to ethanolic extracts. Thus, it was concluded that the ethanolic extract was sensitive to *Mycobacterium tuberculosis* through the resistance ratio method.

The solvent extract of castor (*Ricinus communis* L.) leaves indicated its effect in inhibiting *Mycobacterium tuberculosis* H37RV. *Mycobacterium tuberculosis* H37RV is a type of bacteria that infects human and causes tuberculosis. It is a type of bacteria that is sensitive to first-line drugs, which means that the bacteria can be treated with first-line tuberculosis drugs, such as rifamycin, isoniazid, pyrazinamide, streptomycin, and ethambutol. However, there was a rifampicin-resistant strain (TMC331) of *Mycobacterium tuberculosis* that was obtained from

multidrug-resistant patients (D'Oca et al. 2010, Ladda & Magdum 2012, Ullah et al. 2017, Ladda et al. 2018).

The phytochemical content of castor (*Ricinus communis* L.) leaf extract includes saponins, tannins, alkaloids, flavonoids, and ricinoleic acid (C18:1, OH) that could inhibit the growth of *Mycobacterium tuberculosis* (Kesumasar et al. 2018). Flavonoids were able to inhibit the synthesis of cellular nucleic acids by inhibiting the topoisomerase enzyme of *Mycobacterium tuberculosis*. Flavonoids were also able to inhibit the electron transport chain and ATP synthesis to disrupt the energy source for the cell's life, so that the cell died due to lack of energy (Górniak et al. 2019).

The minimum inhibitory levels of castor (*Ricinus communis* L.) leaf extract were assessed against *Mycobacterium tuberculosis* H37RV, as the bacteria sensitive to first-line drugs, using 200 µg/mL ethanol, 10,000 µg/mL n-hexane, 40,000 µg/mL ethyl acetate, 5,000 µg/mL chloroform, 150 µg/mL acetone, 100 µg/mL methanol, and fatty acid amides dissolved in 12.5 µg/mL dimethyl sulfoxide. Dimethyl sulfoxide extract showed that its MIC worked against *Mycobacterium tuberculosis* H37RV. Dimethyl sulfoxide is an organic solvent that can dissolve polar, non-polar, and semi-polar solvent compounds. Thus, the antitubercular activity only came from the phytochemical agents of castor (*Ricinus communis* L.) leaf extract. It showed that the solvent extract of castor plant (*Ricinus communis* L.) leaf extract had an effect in inhibiting *Mycobacterium tuberculosis* H37RV (D'Oca et al. 2010, Ladda & Magdum 2012, Ullah et al. 2017, Ladda et al. 2018).

Mycobacterium tuberculosis cell wall is composed of a layer of peptidoglycan, which is a complex polymer consisting of a series of N-acetylglucosamine acid and N-acetylmuramic acid arranged alternately. The structure of the cell wall was damaged by inhibiting its formation or by changing it after it was formed. Antimicrobial substances in the form of tannins at low concentrations inhibited the formation of glycoside bonds, so that the formation of cell walls was disrupted. Whereas, antimicrobial substances at high concentrations stopped the formation of cell walls (Maisetta et al. 2019). Alkaloids targeted the DNA topoisomerase I enzyme, inhibited the activity of *Mycobacterium tuberculosis* topoisomerase I (MtbTopoI) and protein synthesis, and single-stranded DNA division that caused cell damage and disrupted the cell's life process (García et al. 2018). The results showed that castor (*Ricinus communis* L.) leaf extract effectively inhibited the activity of *Mycobacterium tuberculosis*.

Research presented in this study only examined the minimum inhibitory levels of castor plant (*Ricinus communis* L.) extract against the growth of *Mycobacterium tuberculosis* H37RV (sensitive to all first-line drugs). Therefore, there is a need for in vitro and in vivo experimental research on the potential of castor (*Ricinus communis* L.) leaf extract against the growth of *Mycobacterium tuberculosis* strains resistant to rifampicin (TMC331) and isoniazid (INH) using the disc diffusion method. The use of dimethyl sulfoxide as solvent is also recommended because it produced a more effective yield of castor plant (*Ricinus communis* L.) leaf extract to inhibit the growth of *Mycobacterium tuberculosis* H37RV (sensitive to all first-line drugs).

Strength and limitation

The Castor leaf extract is one of the innovations in developing new medicines. Exploration of the antimicrobial potential of castor leaf extract (Ricinus communis L.) needs to be carried out further because Castor leaf extract has the potential to inhibit the growth of Mycobacterium tuberculosis, the bacteria that cause TB disease in the future.

CONCLUSION

The analysis of the journal articles using a quantitative systematic review method showed that castor plant (*Ricinus communis L.*) has the potential to inhibit the growth activity of *Mycobacterium tuberculosis*. The extract contains flavonoids, saponins, tannins, alkaloids, and ricinoleic acid compounds that can damage the bonds in bacterial wall, disrupt the formation of energy in the form of ATP, inhibit the topoisomerase I enzyme and protein synthesis, and disrupt the DNA formation. The analysis of the in vitro experiment showed that the use of dimethyl sulfoxide as a solvent of castor plant (*Ricinus communis* L.) leaf extract had minimal inhibitory levels that were effective in inhibiting the activity of *Mycobacterium tuberculosis*.

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Conflict of interest

There were no conflict of interests.

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Author contribution

Fikriaddin Syafiq Istaufa collected the data and wrote this research manuscript. Yoyok Subagio collected the data. Irma Suswati and Isbandiyah checked the result article.

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Literature Review

HEALING IN NURSES AFTER ASSIGNMENT IN NATURAL DISASTERS

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ABSTRACT

Disasters are defined as catastrophic occurrences that impact a large number of people quickly and with an abrupt onset. On average, one natural disaster is reported globally each day. However, the impact of a traumatic occurrence on an individual may be compared to a rock colliding with the surface of a water body. Systematic searches were conducted in Cochrane, Google Scholar, PubMed, Scopus, Embase, and Web of Science databases. The following keywords were used to perform a literature search: "nurse," "healing," and "natural catastrophes". As a result of reviewing 362 abstracts and titles, ten were determined to meet the study goals. The research methods in four of the ten literatures were qualitative, five quantitative, and one was a blend of both. Generally, culturally appropriate psychological first aid sessions, post-natural disaster assignment monitoring, and grit push people to overcome obstacles and accomplish achievements over time. Nurses who have been deployed to natural catastrophes must heal and need time to rest physically and mentally. This study aimed to find out the implications of culturally sensitive psychological first aid sessions, post-assignment in crisis situations monitoring, and grit in pushing individuals to overcome obstacles and achieve success over time; and how the government policies relate to trauma recovery.

Keywords: *Healing; natural disasters; nurse; psychological first aid; human and health; public health*

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Highlights:

1. Disasters impact a large number of people, including nurses who are deployed in the disaster relief.
2. Culturally sensitive psychological first aid sessions, post-assignment in crisis situations monitoring, and grit have implications in pushing the nurses to overcome their trauma and obstacles.
3. Government policies are also important in helping nurses to heal after deployment in disaster relief.

INTRODUCTION

The term "traumatic event" seems to be used interchangeably with the terms "catastrophe" and "very unpleasant event". McFarlane and Norris described a catastrophe as a traumatic occurrence that impacts a large number of individuals quickly and with acute attacks (Nuari 2014). On average, one catastrophe is reported globally each day (Ma et al. 2021). The impact of a traumatic experience on a person, on the other hand, may be compared to a rock striking the surface of a water body. The first collision generates a powerful wave. Then it is followed by a wave that continues to grow in length but diminishes in size (Emily J. Dorosz & Quinn 2019). Similarly, although the effect of a single traumatic experience may be widespread, the ramifications are often less severe in those who are not directly involved in the horrific event (Tyer-Viola 2019).

We think that a traumatic circumstance is not necessarily as terrible for the indirect victims as it is for the direct victims. However, prior research has shown that persons exposed indirectly to traumatic events may develop mental health issues equivalent to those directly exposed, and that proximity to the traumatic event may be a risk factor (Bakker et al. 2021). People around the deceased significantly experience trauma. Other research has shown that losing classmates, friends, and colleagues while serving in the military increases the probability of experiencing persistent anger, guilt, depressive symptoms, and grief. Additionally, Parkes & Prigerson (2013) asserted that the degree of grief is contingent upon one's connection with the wounded or deceased. Other studies also described the link between attachment and loss (Bowlby 1982, Choi 2020). The family bonds and the importance placed on perceiving the family as a whole as well as individuals may serve as a mirror for this devoted community of close relatives.

There is, however, a shortage of research and knowledge of how indirectly exposed nurses interpret the perceived impacts of disaster or trauma on their relationships with survivors or victims' colleagues and friends, as well as their evaluation of post-traumatic military follow-up for nurses in the military. Individuals who are indirectly affected may also be susceptible in the aftermath of a tragedy (Uddin & Matin 2021). Previous research has shown that individuals who have been exposed to traumatic situations develop a mistrust for the organizations to which they belong even though they have to give assistance and provide relief. Their anguish may stem from a sense of injustice and associated negative emotions, such as resentment or rage which may exacerbate other post-traumatic stress responses (Kılıç & Şimşek 2019).

Finally, a qualitative research was undertaken on a sample of indirectly exposed nurses who survived the same avalanche. All of the survivors who were directly exposed in this earlier research noted a lack of help from government institutions immediately after the tragedy and over the next three decades (Bromhead 2021). Therefore, the purpose of this literature review was to conduct a literature study on nurses who are recovering from post-natural disaster assignment.

MATERIALS AND METHODS

This research was a survey of literatures in 2022 to determine the present state of knowledge about nurses' healing post-natural catastrophe assignment. We conducted systematic searches on Cochrane, Google Scholar, PubMed, Scopus, Embase, and Web of Science review databases. The following keywords were used to perform a literature search: "nurse", "healing", and "natural catastrophes". Original peer-reviewed articles, abstracts, reports, and letters to editors written in English and published between 2017 and March 2022 were considered. Non-English articles, ongoing projects, review articles, and publications addressing non-human research were excluded from the literature search. The process for systematic enrollment review was omitted owing to the anticipated scarcity of data and topic requirements. Each of the articles' titles and abstracts was examined, and the most relevant articles were chosen using the previously mentioned inclusion and exclusion criteria. To guarantee the quality of the articles chosen, a checklist consisting of ten categories was established using relevant research. After that, the complete texts of the chosen articles were extensively examined in order to extract significant results.

RESULTS

Literatures that discuss the healing of nurses after being deployed in natural disasters are rare. A combination of primary and secondary search strategies resulted in 362 abstracts which were then screened by reviewers. After a detailed review of the full texts of the selected articles, only about ten were determined to be relevant to nursing, healing, and natural disasters. These articles were discussed according to:

Culturally appropriate psychological first aid sessions

The mean scores assessed the disaster preparedness perception scale among nurses who were trained in the preparation, intervention, and post-disaster phase. The intervention group's mean scores which increased significantly after training and follow-up were higher than those of the untrained group. The intervention group's mean scores for the general self-efficacy assessment also increased significantly after training and follow-up than those of the untrained group.

Follow-up monitoring post-disaster assignment

The governments paid less attention in caring for victims and volunteers after disaster because they are not a priority for them. It resulted in post-disaster unstructed nursing care that made nurses believed they were not treated as the first responders immediately after a natural catastrophe. Following a disaster, victims expressed feelings of abandonment and loneliness, with no important duties to be met. The majority of interviewees underlined the significance of believing in oneself and having strong ties with family, spouse, friends, and peers. These are connections on which they can depend. On the other hand, the majority of victims expressed low institutional confidence in nurses as an organization and unhappiness with the public health system. This was noted by the victims when they expressed their frustration with nurses for ignoring their needs. They stated a pervasive sense of betrayal by nurses and of not being regarded or recognized as the first responder after a crisis.

Grit motivates them to endure challenges and become successful over time

Grit has been equated with the term that best describes the Big-Five personality traits. The Big-Five personality factors were built utilizing approximately 1500 qualities and 10 replications of various factor analytic methodologies, resulting in 75 descriptions of the five factors. Additional study of synonyms for positive and negative traits in the five-factor system revealed that they accurately described five personalities: operation (strong emotional responsiveness to positive influence), sociability, conscientiousness, emotional stability, and intellectuality. Each of these attributes has both good and negative features, so that when analyzed, these attributes may help define a person's personality. Grit is regarded to be a manifestation of deeper awareness

on these attributes. In enumerating the beneficial and detrimental features of awareness, the most highly ranked traits of prudence were organization and efficiency. Indeed, these characteristics are often cited in Florence Nightingale's Nursing Notes and have served as the foundation for the nursing profession (Hammar et al. 2017).

Government policies related to trauma healing

Psychological disorders might develop in catastrophe survivors as a result of trauma. Psychological trauma is described as an episode of strong and unexpected distress that surpasses a person's capacity to bear, deal with, or avoid. After a disaster, survivors are traumatized by the death of a loved one, instilling memories of triggers, such as earthquakes and other natural disasters. After a tragedy, some adult refugees despair, while young refugees are frightened by loud noise and speed at the shelter. Apart from losing loved ones, survivors often lose employment and access to enterprises and finance necessary to sustain themselves. They must be able to promptly stand up and restart everything from zero, even if it means starting from negative circumstances.

DISCUSSION

Culturally appropriate psychological first aid sessions

Cheung (Schafer et al. 2020) stated in a randomized controlled study that psychological first aid training which includes basic disaster interventions is an effective training that equips aid workers with the necessary skills for disaster response preparation. The same research discovered an increase in the intervention group's self-efficacy in giving emotional support to catastrophe survivors. Asman et al. (2020) discovered that psychological first aid training provided to Haitian health personnel increased the confidence in their ability to assist distressed persons in the event of a catastrophe. Prasetiya (2016) discovered through the use of brief questionnaire forms and group interview notes from 76 medical service group volunteers, including nurses (n=27), that psychological first aid training increased their confidence in assisting individuals experiencing psychological distress and preparations for disaster situations.

According to Prasetiya (2016), around 78% of the participants were able to tackle psychological difficulties more readily after a catastrophe as a result of psychological first aid training. Semlitz et al. (Konami et al. 2021) determined that following a training, participants had a significant increase in their ability to assess the needs of people affected by a disaster. They were able to distinguish normal stress reactions from mental health problems, avoid causing harm while assisting people affected by the disaster, and determine what to do next (Inoue et al. 2019). The participants improved their understanding of emergency mental health therapies and their ability to describe acute issues more precisely after completing a consensus-derived empirically validated competency-based psychological first aid training (RAPID–PFA) (Brewer et al. 2020).

Nurses were discovered to be chronically agitated and weary as a result of their recurrent deployments to disaster areas without appropriate mental health recovery chances, such as rest or professional treatment (Jackson & Nowell 2021). Given these findings, it is critical to design evidence-based mental health disaster recovery programs at the national level that include a cultural perspective to assist disaster health care personnel (Uddin & Matin 2021).

Follow-up monitoring of post-disaster assignment

Nurses have several possibilities to be affected by the experience of fellow nurses who have indirectly faced a traumatic incident when it comes to mental health services (Ma et al. 2021, Hopkinson & Jennings 2021). Mental health nursing is characterized in the literature as a patient-centered, professional, goal-directed activity that is evidence-based and focuses on the growth, development, and recovery of individuals with mental health problems (Rustinsyah et al. 2021). It requires compassionate, empathic, perceptive, and courteous nurses to use and develop an individual's own resources and to encourage change in conjunction with people, friends, family, and the health care team/system (Hashim et al. 2021). In military organizations and public health services, the first-person experience is critical for both short- and long-term follow-up. The first person was the individual who was greatly damaged by the occurrence of a catastrophe and badly harmed by loss, grief, guilt, rage, and emotions of being abandoned (Yodsuban & Nuntaboot 2021).

In funerals, the deceased's husbands often get all of the attention, but the deceased's mothers do not (Bromhead 2021). A theory about attachment and loss was described by Parkes & Prigerson (2013) in which bodily and existential anguish can be associated with the death of a close family member or loved one. A study confirmed that the informants' notions about the associaton of physical suffering and the death of close relatives make sense (Razeeni et al. 2021). They want to be viewed as equals to their closest families, as the

first significant other to the deceased or wounded colleagues. In the military, a close relative or colleague wants to be seen as the first person the military system thinks about and acts on. Wright and Leahey, as quoted by Firomsa et al. (2018), examined family theory in relation to nursing by describing each member of the family as a unit separate from the family. Nurses are part a of a group, but also want to be seen as individuals who have their own personalities (Gab Allah 2021). A study by Freitas (2019) found that the informants want to be seen and want their faces to be known. The face is associated with a person's identity because it can emphasize the importance of someone's individuality (Sohrabi et al. 2020). However, in addition to being recognized as relevant individuals who experienced trauma, the nurses want to be recognized as members of their group or organization (Hopkinson & Jennings 2021, Aditya et al. 2021).

Grit motivates them to endure challenges and become successful over time

As previously emphasized, continuous curiosity and perseverance are two distinct components of grit. With enthusiasm, perseverance maintains exceptional performance (Panes et al. 2018). Perseverance is required to cultivate an interest in improving oneself or accomplishing a goal. A critical component of pursuing a goal tenaciously is a feeling of purpose in executing a task (Gab Allah 2021).

A passion is an intense enthusiasm in a certain topic or goal (Hashim et al. 2021). Nursing is sometimes defined as a purposeful profession characterized by a strong commitment to patient care. Nursing's primary concern is to put patients in the greatest possible situation for healing, as Florence Nightingale advocated (Yodsuban & Nuntaboot 2021). Nurses' acts amid a catastrophe demonstrate their commitment and purpose. The nursing team's ability to evacuate patients from hospitals is critical. Evacuations are still an uncommon occurrence, and when they occur, they lack true emergency status (Uddin & Matin 2021). During Hurricane Sandy, nurses were tasked with the responsibility of implementing an evacuation strategy on a shoestring budget. Nurses understand the critical nature of this job and depend on one another to survive and fulfill the challenging duty (Yusuf et al. 2020b). Nurses demonstrate fortitude through their ability to get by and work together to address challenges. Emergency preparedness is contingent on nurses and transdisciplinary colleagues performing admirably under all circumstances (Uddin & Matin 2021). Grit development among nurse leaders and clinical nurses is crucial for sustaining interest in emergency preparation and tenacity during times of crisis. “Grit: The power of passion and perseverance” discusses important leaders and organizational activities that will help growing a productive workforce during and after major events (Yusuf et al. 2020a).

Government policies related to trauma healing

Values in society are critical for expediting the trauma recovery process (Endang et al. 2022). The strategies proposed for expediting the trauma recovery process are as follows. First, strengthening the capacity of the psychiatrist/counselor/therapist resources: A counselor should be able to give trauma counseling services by establishing a feeling of security and providing individual counseling via the use of systematic desensitization procedures followed by release techniques (Rohmi et al. 2020). Second, involving family members and students in the trauma recovery program. The rehabilitation process is influenced by environmental, individual, and event-related social variables (Schafer et al. 2020). Parents, as the head of the family, must understand and acquire trauma management skills in order to monitor and assist children and other family members in coping with trauma, while school personnel such as teachers and administrators may aid in the trauma recovery process. It is envisaged that via education, catastrophe risk reduction activities may reach larger goals and can be presented to all learners at an early age in order to contribute to individual and community preparation (Amberson et al. 2020). Third, improving the coordination of community-based institutions. By involving various stakeholders, particularly teachers and volunteer community leaders from non-governmental organizations (NGO) engaged in the field of trauma healing, local governments can commit to and hopefully coordinate synergistically with the National Disaster Relief Agency, the Office of Education, and the Office of Health (Ma et al. 2021). Fourth, mentoring disaster victims. The mentoring activity is to provide recovery to individuals experiencing crisis problems through crisis intervention, individual counseling and, if necessary, group counseling, as well as prevention efforts to prevent those with trauma (their fellow victims) from mutually providing support assistance. Additionally, it plays a role in regulating the psychological development of catastrophe victims after trauma recovery (Kılıç & Şimşek 2019). Fifth, increasing social, religious, and cultural values throughout the trauma healing process. During the trauma healing process, the leaders or figures internalize social, religious, and cultural values and signify the role of the community. Tucking these beliefs in may provide social support for catastrophe survivors, which is critical for post-traumatic development (Uddin & Matin 2021, Vaitheswaran et al. 2020). Sixth,

combining pharmaceutical and psychotherapeutic treatment for catastrophe victims. Pharmacotherapy is a term that refers to the use of medicine as a therapeutic aid in the psychological treatment of catastrophe victims (especially for victims who have experienced trauma or stress) (Uddin & Matin 2021). This treatment facilitates psychotherapy for the sufferers of catastrophic illness or injuries via relaxation. However, since the introduction of the analytic or medicine may result in unabated side effects and sensations of pain, as well as the risk of problems, such as dependency, nausea, vomiting, and constipation, a safer intervention is required (Eroy 2021). Seventh, the use of non-pharmacologic treatment that is aimed at disaster victims and customized to their specific requirements (Ma et al. 2021). Non-pharmacological action is a kind of treatment that promotes trauma recovery without the use of pharmaceuticals, but rather through physical and cognitive approach. The rehabilitative program is available in a variety of formats but must be customized to the demands of the target population or catastrophe victims (Stoklosa et al. 2021). Eighth, ongoing and routine trauma healing program development. Trauma healing program is developed in order to undertake monitoring and assessment of psychological or trauma-related injuries sustained by catastrophe victims. This program will continue until all catastrophe victims are entirely healed (Bakker et al. 2021).

Strength and limitation

The study used a combination of qualitative and quantitative research methods, which can provide a more complete understanding of the topic.The study reviewed a relatively large number of abstracts and titles (362), which suggests that the study team made a thorough effort to identify relevant studies.This study identified specific interventions (culturally appropriate psychologica first aid sessions, post-disaster assignment monitoring, and grit) that may be useful for individuals recovering from natural disasters.and also addresses the need for nurses who have been deployed to natural disasters to have time to rest and heal.

CONCLUSSION

Nurses who have been sent to natural catastrophes must heal and need time to rest physically and mentally. Implications for culturally sensitive psychological first aid sessions, post-assignment monitoring in crisis situations, and grit push individuals to overcome obstacles and achieve success over time, while government policies also have an implication in affecting trauma recovery.

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Conflict of interest

No conflict of interest has been declared.

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Author contribution

FKS and RSA conceived the idea of the study. FKS prepared the draft of the manuscript. RSA was in charge of the manuscript arrangement. All authors were involved in the revision of the manuscript and have agreed to the final content.

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Meta-Analysis

OPTIMAL DOSE OF VITAMIN D FOR COVID-19 TREATMENT

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ABSTRACT

This meta-analysis aimed to determine whether there is any optimal dose of vitamin D for morbidity, length of hospitalization, and mortality in patients with COVID-19. We conducted a comprehensive search in three online databases for eligible studies until February 28, 2022. Odds ratio (OR) and standardized mean difference (SMD) were applied as summary statistics of primary outcomes. The study quality of the literatures collected was assesed using the Cochrane risk of bias tool version 2 (RoB 2). Eight randomized clinical trials (RCT) were included in the study. In our analysis, we found that there was no significant difference in morbidity when vitamin D was administered to COVID-19 patients [OR=0.50 (95% CI=0.13-1.96); SMD=-0.14 (95% CI=-0.55-0.28)]. Duration of hospitalization [SMD=-0.12 (95% CI=-0.39-0.15)] and mortality [OR 0.47 (95% CI=0.19-1.17)] of COVID-19 patients in five studies also showed no significant difference compared to patients who did not take vitamin D. However, when we analyzed two other studies, we found that in patients who did not take vitamin D, mortality was lower [SMD=0.43 (95% CI=0.29, 0.58)]. In conclusion, compared to a single high dose of vitamin D, the multi-day vitamin D administration of 1000-6000 IU in patients with COVID-19 resulted in improved patient morbidity, length of hospitalization, and patient mortality.

***Keywords**: Vitamin D therapy; COVID-19; morbidity; mortality; hospitalization; infectious disease*

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Highlights:

1. Vitamin D supplementations in different doses yield different outcomes.
2. Multi-day vitamin D administration of 1000-6000 IU in patients with COVID-19 has more positive impacts than a single high dose of vitamin D.
3. Patient morbidity, length of hospitalization, and patient mortality improved with multi-day vitamin D administration.

INTRODUCTION

The coronavirus (COVID-19) pandemic remains a public health concern. COVID-19 is caused by the SARS-CoV-2 virus, which belongs to the coronavirus family. This virus is highly contagious and is spreading rapidly worldwide (Rawaf et al. 2020, Centers for Disease Control and Prevention (CDC) 2022). COVID-19 has undergone many mutations and has given rise to several variants. The most recent study revealed the discovery of the most recent variant of COVID-19, the omicron variant (B.1.1.529), which was first identified in early November of 2021 in Botswana (Gao et al. 2022).

As research on COVID-19 progresses, many treatments are now available to treat COVID-19 that have been approved by the Food and Drug Administration (FDA), ranging from antiviral drugs, monoclonal antibodies against SARS-CoV-2, anti-inflammatory drugs, and immune-modulating drugs (Cascella et al. 2022). As with other viral diseases, COVID-19 treatment remains primarily supportive care to correct the patient's condition, as there is no definitive treatment for COVID-19 that is highly effective (Stasi et al. 2020). Based on Sutadji JC et al. (2021), 10 of the 12 available studies found lower mortality when using anti-IL-6 therapy compared with standard care, although lower mortality in patients Larger randomized controlled trials were needed to clarify the efficacy of anti-IL-6 therapy in severe COVID-19. Research continues to develop optimal treatments that can increase the COVID-19 recovery rate, including the use of vitamin D in the treatment process (Sánchez-Zuno et al. 2021, Sabico et al. 2021).

Vitamin D is known to play a key role in controlling the immune system, including protection against viral infections. Vitamin D deficiency can increase the severity of influenza and respiratory infections (Grant et al. 2020). Activation of vitamin D receptors on immune cells has shown direct effect by reducing the secretion of inflammatory cytokines, such as IL-6, and indirect effect through C-reactive protein (Ohaegbulam et al. 2020). Vitamin D supplementation has been suggested as a possible way to prevent infection, severity of illness, and death from the disease (Brenner 2021). Several studies with varying outcomes have demonstrated the differences in vitamin D levels between healthy individuals and COVID-19 patients, and the impact of vitamin D deficiency on the risk of developing COVID-19 and its complications. High doses of vitamin D (200,000 IU) once were not found to significantly shorten treatment duration. Meanwhile, administration of 60,000 IU vitamin D for 8 to 10 days significantly reduced inflammatory markers associated with COVID-19 without adverse effects. Two weeks of 5,000 IU vitamin D adminisitration has been shown to shorten recovery time from ageusia and cough in patients with mild to moderate symptoms of COVID-19. Another study found that a dose of 10,000 IU vitamin D for two weeks improved the patient's clinical condition (Murai et al. 2021a, Sánchez-Zuno et al. 2021, Sabico et al. 2021, Lakkireddy et al. 2021).

The existence of different outcomes from treatment with vitamin D leaves the debate unresolved regarding the optimal dose of vitamin D in COVID-19 patients. This study aimed to determine if there is an optimal dose of vitamin D for morbidity, duration of hospitalization, and mortality associated with COVID-19.

MATERIALS AND METHODS

This systematic review and meta-analysis were carried out based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines in a study by (Page et al. 2021). We conducted a digital data search for relevant studies published up until February 28, 2022 in PubMed, Scopus, and the Cochrane Central Register of Controlled Trials (CENTRAL). These search terms were entered: ("COVID-19" OR "COVID19" OR "SARS-CoV-2" OR "SARSCoV2" OR "SARS-Cov-19" OR "SARSCoV19" OR "2019-nCoV" OR "2019nCoV" OR "nCoV-2019" OR "nCoV2019" OR "coronavirus disease 2019" OR "novel coronavirus" OR "new coronavirus") AND ("vitamin D" OR "vitamin D3" OR "vitamin D dosage" OR "vitamin D therapeutic use" OR "vitamin D therapy") AND ("morbidit*" OR "mortalit*" OR "death*" OR "hospitalization*" OR "hospitalisation*" OR "severity"). No publication date restrictions were set in all searches.

After removing the duplicates, the remaining articles were filtered by reviewing their titles. Abstracts of some articles that have relevant possibilities are further filtered. Lastly, the selected articles with available full-texts were retrieved and assessed according to the eligibility requirements. Two investigators (DMU and MARA) independently accomplished the overall study selection process. Disagreements were discussed with the other investigators until consensus was reached.

We included all studies investigating the association of vitamin D administration (by any definition) with COVID-19 morbidity, length of hospitalization, and mortality in populations aged 18 years or older. Exclusion criteria were: 1) irrelevant titles or abstracts; 2) irretrievable full-texts; 3) review articles, case reports, observational studies, case series, conference abstracts, or letters to editors; 4) non-English studies; or 5) insufficient data to calculate the effect sizes for all outcomes (COVID-19 morbidity, length of hospitalization, and mortality).

Out of three authors, two (DRR, MIM) separately extracted all the data, and then the third author (HTAF) double-checked its accuracy. Discussions were used to settle disagreements. The following relevant informations were gathered for each study that included the first author, publication year, study location, study design, COVID-19 diagnosis definition, the dosage of vitamin D administration, population age, female percentage, the sample size in each group (vitamin D group vs control), and vitamin D effects on patients with COVID-19 (morbidity, length of hospitalization, and mortality).

With the Cochrane RoB tool version 2 (RoB 2), two authors (MZT and JNS) independently evaluated the methodology quality from each study. The following five domains of observational studies were evaluated: 1) the randomization method; 2) deviations from the intended interventions; 3) missing result or outcome data; 4) outcome measurement; and 5) reported result choice. The signaling questions have five possible answers: No, Probably No, Probably Yes, Yes, and No Information. A recommended risk-of-bias judgment for every domain was mapped onto responses to signaling questions using algorithms included in the program. For each domain, there were three alternative risk-of-bias assessments: 1) low risk of bias; 2) some concerns; and 3) high risk of bias. When research is deemed to have a low risk of bias across all domains, the overall risk of bias assessment is low. Some concerns are assesed if the study is determined to raise some concerns in at least one domain but not to be highly biased in any domain. High risk of bias means that there is one domain or more where the study is considered to have a high risk of bias for this research result or there are numerous domains which have some concerns that significantly reduce confidence in the result.

that there is one domain or more where the study is considered to have a high risk of bias for this research result or there are numerous domains which have some concerns that significantly reduce confidence in the result.

All analyses were conducted using the Review Manager version 5.4 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark). Primary analyses were conducted to evaluate the association between vitamin D administration with three different outcomes related to COVID-19: morbidity, length of hospitalization, and mortality. The OR and standard mean difference were applied as the summary statistics of primary outcomes. Meta-analysis for each outcome was conducted only if there were 2 or more studies reporting the same type of data. All analyses were performed both with and without outliers. A study was considered an outlier when its 95% confidence interval (CI) was outside the 95% CI of the pooled effect size. Outliers were identified by visually inspecting the forest plots.

The assessment of statistical heterogeneity between studies used I^2, with significance at $p < 0.05$. A random-effect or fixed effect model will be selected based on the value of I^2 to assess pooled standardized mean differences (SMD) and pooled odds ratio (OR). When the value of $I^2 > 50\%$, a random-effect model should be used. Meanwhile, the fixed-effect model will be used if the value of $I^2 < 50\%$. A qualitative assessment of publication bias was carried out using a funnel plot.

RESULTS

Preliminary search of three databases (PubMed, Scopus, and Cochrane) using pre-compiled keywords yielded 1751 studies. The researcher also conducted a manual search and found a study that was not filtered in a keyword search. Of all these studies, there were 538 duplications, leaving 1214 studies to be screened for titles and abstracts. At the first screening, title discrepancies were found in 1117 studies and abstract discrepancies in 37 studies. The subsequent screening revealed the absence of full-text in 26 studies and one non-English study. In the eligibility assessment, there was 1 study with a population discrepancy based on the inclusion criteria, 1 study with an outcome that was not of interest to the study, and 22 studies with an inappropriate study design. In the end, 9 studies that were found met the eligibility criteria for a systematic review and 8 studies that could be analyzed were also processed quantitatively (Quesada-Gomez et al. 2020, Murai et al. 2021a, 2021b, Sánchez-Zuno et al. 2021, Beigmohammadi et al. 2021, Maghbooli et al. 2021, Soliman et al. 2022, Cannata-Andía et al. 2022). Overall this process is shown in the PRISMA flow chart (Figure 1).

The characteristics of nine randomized clinical trials (RCT) studies are summarized in Table 1. More than a thousand of participants (mean age: 48 to 58.5 years) were successfully collected from the included studies (Quesada-Gomez et al. 2020, Murai et al. 2021a, 2021b, Sánchez-Zuno et al. 2021, Sabico et al. 2021, Beigmohammadi et al. 2021, Maghbooli et al. 2021, Soliman et al. 2022, Cannata-Andía et al. 2022). Female participants accounted for approximately half of the overall study population. The diagnostic definition of COVID-19 in all studies was by reverse transcriptase polymerase chain reaction (RT-PCR) tests. Vitamin D administration varied among studies. All studies were carried out on four major continents: America, Europe, Africa, and Asia. One of the studies was conducted in four countries and two continents (Cannata-Andía et al. 2022). Four studies involved similar participants in different numbers (Murai et al. 2021a, 2021b, Beigmohammadi et al. 2021, Maghbooli et al. 2021). The quality of each study assessed using RoB 2 is shown in Figure 2 and Figure 3. Two studies were determined to be at risk of bias (Sánchez-Zuno et al. 2021, Sabico et al. 2021), one study had some concerns (Beigmohammadi et al. 2021), and five other studies were at low risk of bias (Quesada-Gomez et al. 2020, Murai et al. 2021a, 2021b, Maghbooli et al. 2021, Soliman et al. 2022, Cannata-Andía et al. 2022).

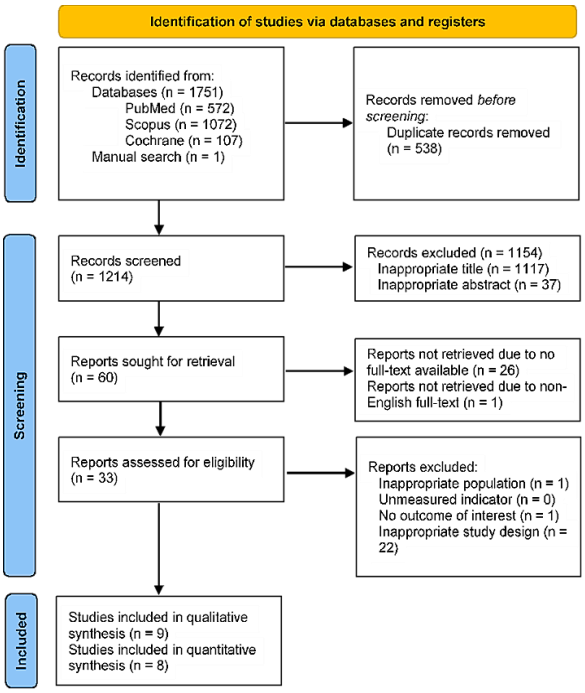

Figure 1. PRISMA flow chart of the study selection process (Page et al. 2021)

Analysis of five studies involving more than 300 subjects showed that administration of vitamin D offered no significant difference in COVID-19 morbidity compared to the groups who did not receive vitamin D as adjunctive therapy (Quesada-Gomez et al. 2020, Murai et al. 2021b, Sánchez-Zuno et al. 2021, Maghbooli et al. 2021, Soliman et al. 2022). However, the administration of vitamin D tended to have a protective effect in the COVID-19 morbidity [OR=0.50 (95% CI=0.13-1.96)] (Figure 4).

Table 1. Characteristics of the included studies

Author & Year	Study Location	Study Design	COVID-19 Diagnostic Definition	Vitamin D Administration	Age of Patients	Total Patients (% female)
Murai et al. (2021a)	Brazil, America	RCT	Nasopharyngeal swab PCR or CT scan	A single dose of 200,000 IU vitamin D3 dissolved in a 10-mL peanut oil solution by oral.	56.2±14.4*	273 (43.9)
Cannata-Andía et al. (2022)	Spain, Europe; Argentina, America; Guatemala, America; Chile, America	RCT	Nasopharyngeal swab with RT-PCR or antigen tests	A single oral bolus of 100,000 IU cholecalciferol	58.0**	543 (42.9)
Maghbooli et al. (2021)	Iran, Asia	RCT	RT-PCR and CT scan data from medical records	25,000 IU vitamin A given daily, 600,000 IU vitamin D given once during the study, 300 IU vitamin E given twice a day, 500 mg vitamin C given four times a day, and one daily ampule of B vitamins taken as Soluvit for 7 days	49.1±14.1*	106 (39.6)
Murai et al. (2021b)	Brazil, America	RCT	PCR or ELISA	Single dose of 200.000 IU vitamin D3	58.5±15.6*	32 (53.1)
Quesada-Gomez et al. (2020)	Spain, Europe	RCT	PCR	Oral calcifediol (0.532 mg) given on hospital admission. The treatment group continued with oral calcifediol (0.266 mg) on days 3 and 7, and then weekly until discharge or ICU admission	53±10*	76 (41)
Soliman et al. (2022)	Egypt, Africa	RCT	RT-PCR	A single dose of 200.000 units vitamin D given intramuscularly	NA	56 (NA)
Sánchez-Zuno et al. (2021)	Mexico, America	RCT	PCR	Daily supplementation of 10,000 IU vitamin D3 in soft capsule form for 14 days	43.0 (20-74)**	42 (52.3)
Beigmohammadi et al. (2021)	Iran, Asia	RCT	RT-PCR and CT scan data from medic records	25,000 IU vitamin A daily, 600,000 IU vitamin D once during the study, 300 IU vitamin E twice a day, 500 mg vitamin C four times a day, and a daily ampule of B vitamins taken as Soluvit for 7 days	52.00 (9.00)**	60 (48.4)
Sabico et al. (2021)[x]	Kingdom of Saudi Arabia, Asia	RCT	RT-PCR	Standard vitamin D therapy 1000 IU (control) or 5000 IU vitamin D3 for 14 days	49.8±14.3*	69 (52.2)

COVID-19, Coronavirus Disease 2019; IU, International Unit; RCT, Randomized Clinical Trial; RT-PCR, reverse transcriptase-polymerase chain reaction; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SD, standard deviation.
* Age of patients are provided in mean±SD
** Age of patients are provided in median (IQR) [x] study that is not included in the quantitative analysis

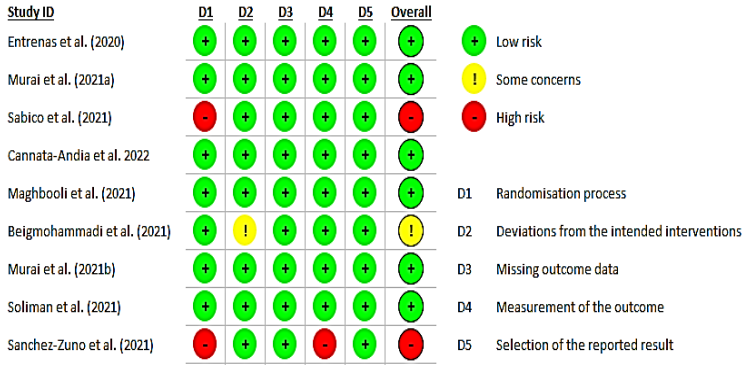

Figure 2. The assessments of study quality using Risk of Bias tool version 2

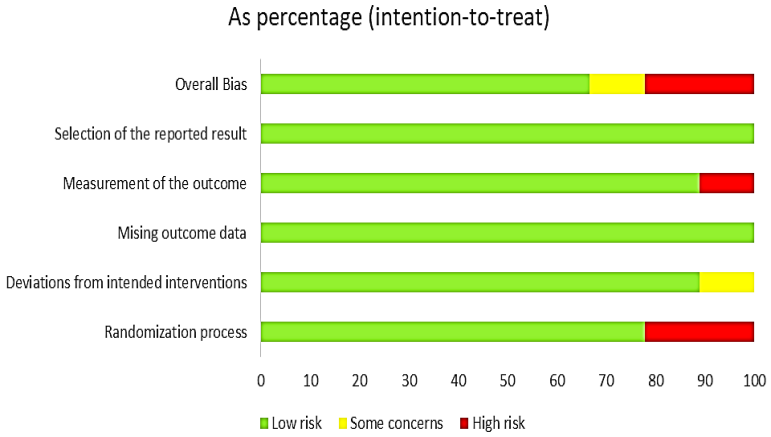

Figure 3. The overall bias of the study quality assessment as a percentage

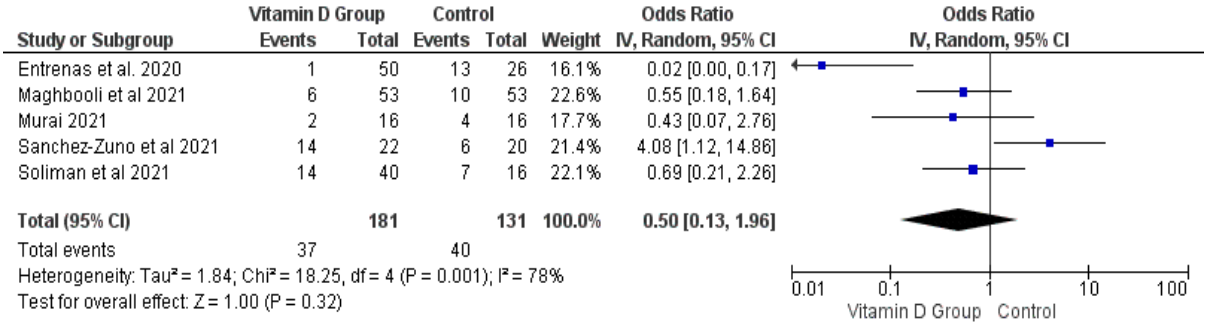

Figure 4. Odds ratio of vitamin D administration on the morbidity in patients with COVID-19

Meanwhile, the pooled analysis of the other two studies involving more than 700 subjects showed that vitamin D administration did not significantly reduce the morbidity in patients with COVID-19 [SMD=-0.14 (95% CI=-0.55-0.28)] (Murai et al. 2021a, Cannata-Andía et al. 2022) (Figure 5). The heterogeneities of all latter analyses were considered high (I^2>50%). This was a possible bias from publications with asymmetric funnel plot results. (Figure 6), either for the first five studies or the other two studies, on the morbidity in COVID-19 patients.

Four studies involving a total of approximately 200 subjects were further analyzed to determine the combined yield of vitamin D administration in the hospitalization day of patients with COVID-19 (Murai et al. 2021a, 2021b, Maghbooli et al. 2021, Cannata-Andía et al. 2022) (Figure 7).

Administering vitamin D in patients with COVID-19 did not significantly reduce the hospitalization day compared to patients not administered with vitamin D [SMD=-0.12 (95% CI=0.39-0.15)]. The heterogeneities between studies in the analysis were low (I^2=0%). The results of the funnel plot on the day of hospitalization in patients with COVID-19 revealed a tendency of no potential publication bias.

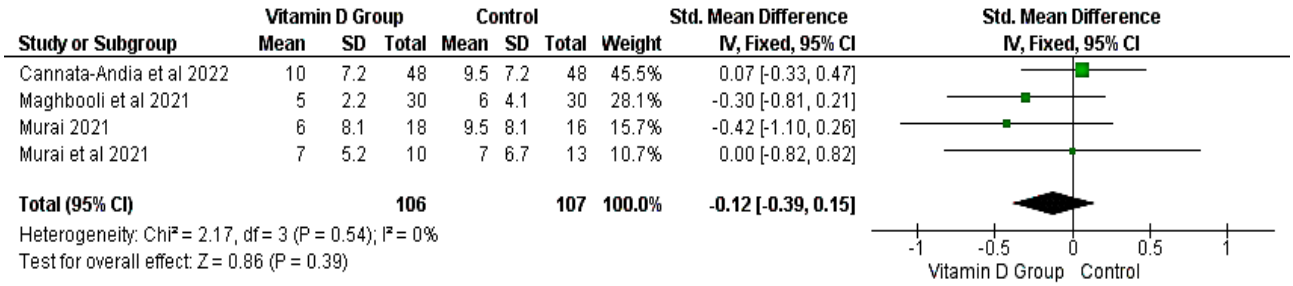

Figure 5. The standard mean difference of vitamin D administration on the morbidity in patients with COVID-19

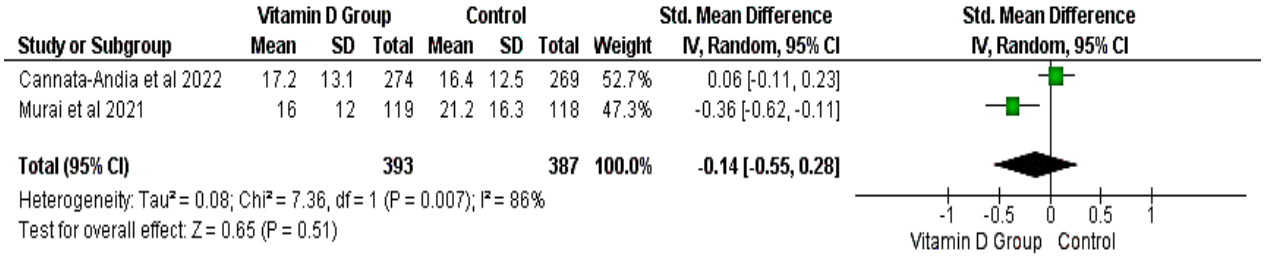

Figure. 6. Funnel plots of the selected studies

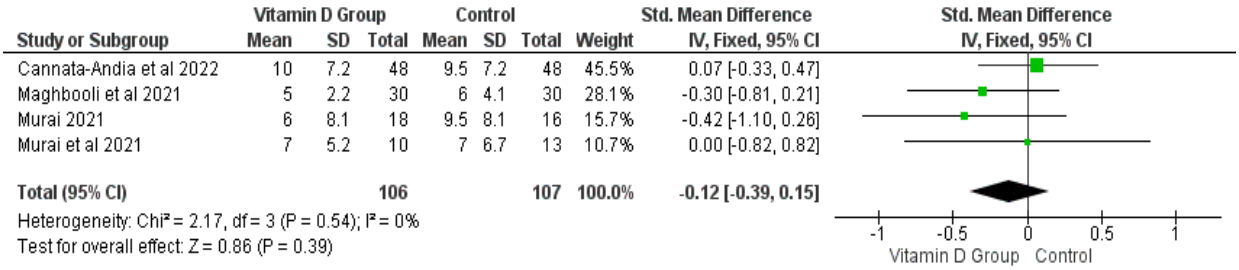

Figure 7. The standard mean difference of the effect of vitamin D administration on the hospitalization day in patients with COVID-19

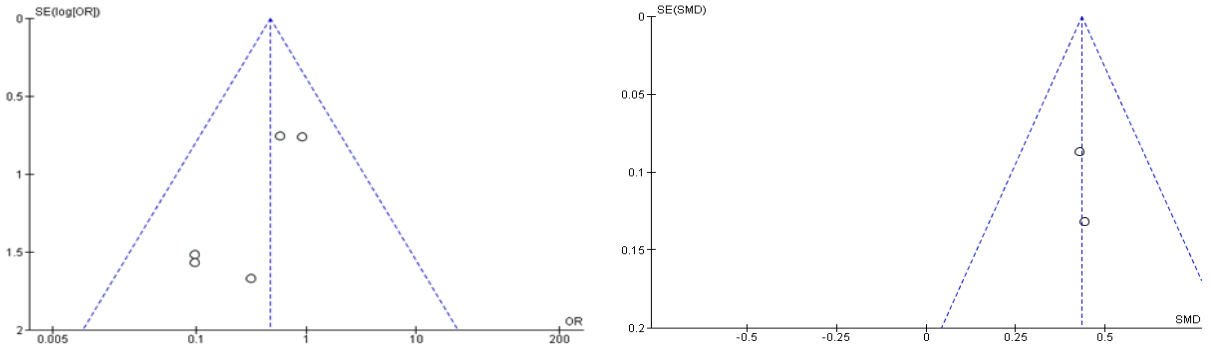

Figure 8. Funnel plot of the effect of vitamin D administration on the hospitalization day in patients with COVID-19

Results from five studies involving approximately 300 COVID-19 patients can be seen in Figure 9 (Quesada-Gomez et al. 2020, Murai et al. 2021b, Beigmohammadi et al. 2021, Maghbooli et al. 2021, Soliman et al. 2022). The OR was 0.47 (95% CI=0.19-1.17) that indicated vitamin D administration tended to have effectiveness in preventing from COVID-19 death. The heterogeneity of the latter analysis was considered low (I^2=0%). However, in the analysis of the other 2 studies, there was a significant difference, in which control patients without vitamin D administration had a low mortality rate [SMD=0.43 (95% CI=0.29-0.58)] with negligible heterogeneity (I^2=0%) (Figure 10). The funnel plot in the COVID-19 mortality indicated the analysis results had a potential bias, while the second analysis indicated a tendency of no publication bias (Figure 11).

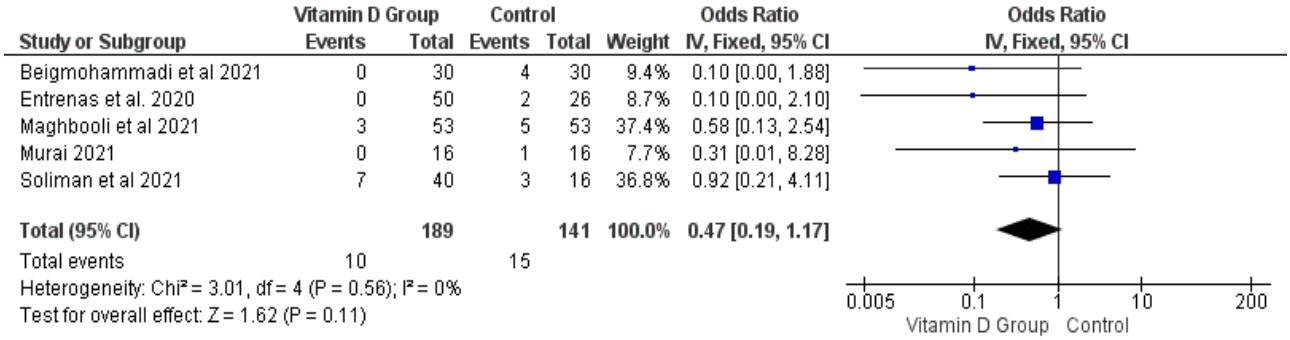

Figure 9. Odds ratio of the effect of vitamin D administration in patients with COVID-19

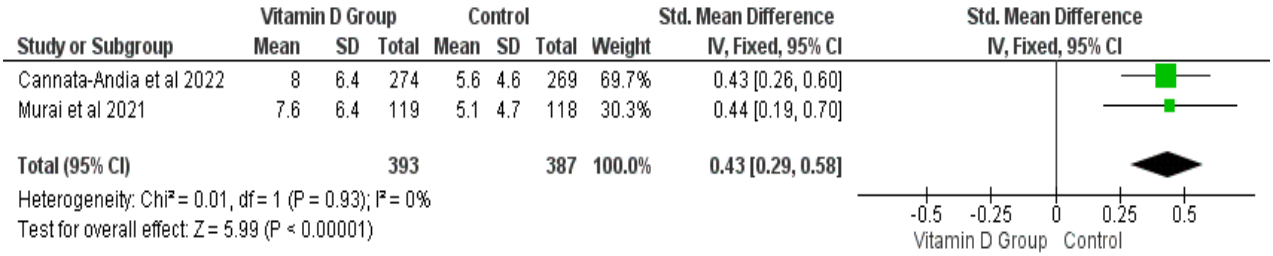

Figure 10. Standard mean difference of the effect of vitamin D administration in COVID-19 mortality

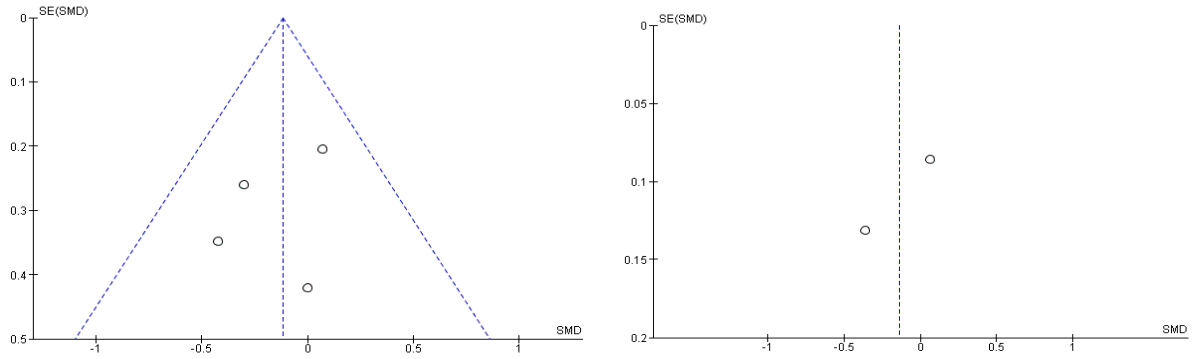

Figure 11. Funnel plots of the selected studies

DISCUSSION

Several studies suggest the prevention or treatment of COVID-19 using vitamin D because it can improve its insufficiency or deficiency, so that it can increase the percentage of blood lymphocytes and improve immune function (Mitchell 2020, Martineau & Forouhi 2020, Malaguarnera 2020, Maghbooli et al. 2021). This systematic review and meta-analysis aimed to determine the optimal dose of vitamin D administration that affect the morbidity, length of hospitalization, and mortality in COVID-19 patients. From this study, the administration of vitamin D did not provide a significant difference in the morbidity of COVID-19 patients compared to the ones who were not given vitamin D as an adjunct therapy. However, it tended to have a protective effect in the COVID-19 morbidity [OR=0.50 (95% CI=0.13-1.96)]. Meanwhile, the pooled analysis of the other two studies involving more than 700 subjects showed that vitamin D administration did not significantly diminish the morbidity in COVID-19 patients [SMD=-0.14 (95% CI=-0.55-0.28)] (Murai et al. 2021a, Cannata-Andía et al. 2022). According to Maghbooli et al., this may happen due to organ damage related to the cytokine storm.

When organ damage occurs, it is difficult to change, so that a more rapid increase in serum concentrations of 25(OH)D3 could be advantageous in diminishing morbidity associated with infectious diseases such as COVID-19 (Maghbooli et al. 2021). The study by Sánchez-Zuno et al. (2021) proved that outpatients with vitamin D deficiency had more COVID-19 symptoms than patients with vitamin D deficiency. This is contrast to the study by Cannata-Andía et al. where there was not a significant difference in COVID-19 outcomes between patients who were given a single oral bolus of cholecalciferol at admission and those who were not given one (Cannata-Andía et al. 2022). According to Soliman et al. and Maghbooli et al., taking vitamin D supplements did not have a significant difference in diminishing the risk or severity of SARS-CoV-19 and the placebo group (Maghbooli et al. 2021, Soliman et al. 2022). The research in Brazil also supports the claim that in moderate to severe COVID-19 hospitalization, cholecalciferol administration did not diminish the use of mechanical ventilation and ICU admission because 25-hydroxyvitamin levels increase rapidly (Murai et al. 2021a).

The length of hospitalization stay is one indicator of clinical developments assessment in COVID-19 patients. Giving vitamin D supplementation to COVID-19 patients is considered to be able to reduce the length of hospitalization stay (Carpagnano et al. 2021). In our findings, administering vitamin D was not significantly reduce the hospitalization day in COVID-19 patients [SMD=-0.12 (95% CI=-0.39-.15)] compared to the patients who did not receive vitamin D. A study by Cannata-Andía et al. (2022) showed that a single oral bolus of 100,000 IU cholecalciferol given at admission did not improve disease outcomes, including the length of hospitalization, compared to patients who did not receive it. Similar results were obtained in the other two studies conducted by Murai et al., where the administration of a single high doses (200,000 IU) vitamin D did not significantly reduce the length of stay in hospital both in the population of COVID-19 patients with non-severe and severe 25(OH)D deficiency (Murai et al. 2021a, 2021b). Contradictory results were demonstrated in a study by Maghbooli et al. (2021) which showed that oral consumption of calcifediol for 60 days at a dose equivalent to 3000-6000 IU vitamin D3 per day proved to be safe and effective in maintaining optimal serum 25(OH)D3 concentrations. Optimal 25(OH)D3 serum in the body has potential benefits to improve immune function by increasing the percentage of lymphocytes and can reduce the length of hospitalization stay in COVID-19 patients (Maghbooli et al. 2021).

Our findings indicated that vitamin D administration tended to have a protective effect from COVID-19 mortality [OR=0.47 (95% CI=0.19-1.17)] although it was not significantly different compared to patients who did not receive vitamin D. However, in an analysis of two other studies found a significant difference where control patients who did not receive vitamin D had a lower mortality rate (Murai et al. 2021a, Cannata-Andía et al. 2022). This occured due to several factors that can be biased, such as heterogeneous recruited population, more prevalence of hypertension, diabetes, and obesity in the group receiving vitamin D, and other influencing factors such as age and current illness (Murai et al. 2021a, Cannata-Andía et al. 2022). From another study, it was also stated that giving high doses of vitamin D for two weeks also did not affect the COVID-19 mortality (Sabico et al. 2021). The mode of administration and dosage of vitamin D in COVID-19 is currently still under controversy (Camargo et al. 2020, Mazess et al. 2021, Pal et al. 2022).

Many studies have proven that vitamin D is useful in treating COVID-19 patients. Nevertheless, the problem is that there is still no agreed optimal dose to guide the administration of vitamin D therapy in COVID-19 patients (Vimaleswaran et al. 2021). Various clinical trials have been conducted as an effort to determine the effect of giving vitamin D therapy at various doses on the outcome of COVID-19 patients. The different findings in these studies were explained by discussing the differences in vitamin D administration in each study. The basic differences in these studies were the

doses and durations of vitamin D administration. Studies with continuous vitamin D administration over a period of time have shown more promising results in clinical outcomes of COVID-19 patients. This is due to vitamin D levels that can be maintained stably and longer in the body compared to single-dose vitamin D administration (Apaydin et al. 2018). This was also found in the study by Sabico et al. where they compared the administration of 1000 IU and 5000 IU vitamin D in COVID-19 patients and for 14 days each. The study demonstrated clinically significant improvement in patients receiving 5000 IU vitamin D compared to patients receiving1000 IU (Sabico et al. 2021).

Strength and limitation

The study assessed the quality of the literature collected using the Cochrane risk of bias tool version 2 (RoB 2), which is a standardized tool for assessing the risk of biasin randomized controlled trials (RCTs). The study included eight RCTs, which can provide a higher level of evidence compared to other study designs. Our study has several limitations.

The main limitation of this study lies in the misalignment of the definition of morbidity used as the desired outcome of the study. Morbidity as a study outcome varies from the duration of sitalization, the severity of conditions associated with medical intervention, and worsening of disease symptoms. Another limitation is the presence of variables other than vitamin D administered to patients that allow for biased results. In addition, some studies also contain data collected from many centers.

CONCLUSION

In conclusion, COVID-19 patients who are given vitamin D as adjunctive therapy tends to have lower but not significantly lower COVID-19 morbidity and mortality when compared to those who did not receive vitamin D. Continuous administration of vitamin D with a dose of 1000-6000 IU for several days in COVID-19 patients has shown better benefits on the morbidity, length of hospitalization stay, and mortality than a single high dose vitamin D. However, further study is still needed to find out which vitamin D dose given to COVID-19 patients is better than the other by comparing them one by one.

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Conflict of interest

There was no conflict of interest in this research.

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Author contribution

All author were responsible for the manuscript preparation. DMU was responsible for conceptualization, data collection, and providing analysis. MARA was responsible for investigation of the data, data collection, manuscript revision, and grammatical checks. DRR was responsible for investigation of the data, data collection, and providing data analysis. MIM was responsible for investigation of the data, data collection and providing data analysis. MZT was responsible for data collection and data analysis. JNS was responsible for data collection and investigation of the data. HTAF was responsible for data collection and providing data analysis. BU was responsible as the supervisor, corresponding author, and also involved in manuscript preparation and validation. SF was responsible for supervision and manuscript preparation. All authors read and approved the final manuscript. Data sharing was not applicable.

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On the other hand, **Invited Literature Review** provides a detailed and comprehensive narrative analysis of recent developments in a specific topic in medicine and highlights important points that have been previously published. The text consists of Abstract, Introduction, highlights, any subheadings as needed by the author(s), Strength and limitations, Conclusion, Acknowledgement, Conflict of Interest, Funding Disclosure, Author Contribution, and References. The text is relatively long compared to other paper categories, typically up to 15 manuscript pages or 4,000 words with approximately 30-50 reference list to comprehensively cover all the major published work.

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1. Journal

Up to three authors, list all the authors.

Halpern SD, Ubel PA, Caplan AL (2002). Solid-organ transplantation in HIV-infected patients. N Engl J Med 347, 284-287 (doi: …….)

More than three authors, list the first three authors, followed by et al.

Rose ME, Huerbin MB, Melick J, et al (2002). Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res 935, 40-46 (doi: …….)

2. Book

Two to three authors, list all the authors.

Sambrook J, Russerl DW (2001). Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, New York.

More than three authors, list the first three authors, followed by et al.

Reece JB, Lisa AU, Peter VM, et al (2010). Campbell biology. Pearson, London.

Chapter in a book

Meltzer PS, Kallioniemi A, Trent JM (2002). Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW (eds). The genetic basis of human cancer, New York, McGraw-Hill, p 93-113.

Electronic book/ E-book

Chapter from an electronic book

Darwin C. On the origin of species by means of natural selection or the preservation of favoured aces in the struggle for life [Internet]. London: John Murray; 1859. Chapter 5, Laws of variation. [cited 2010 Apr 22]. Available from: http://www.talkorigins. org/faqs/origin/ chapter5.html.

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3. Proceeding

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5. Homepage/ Website

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

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