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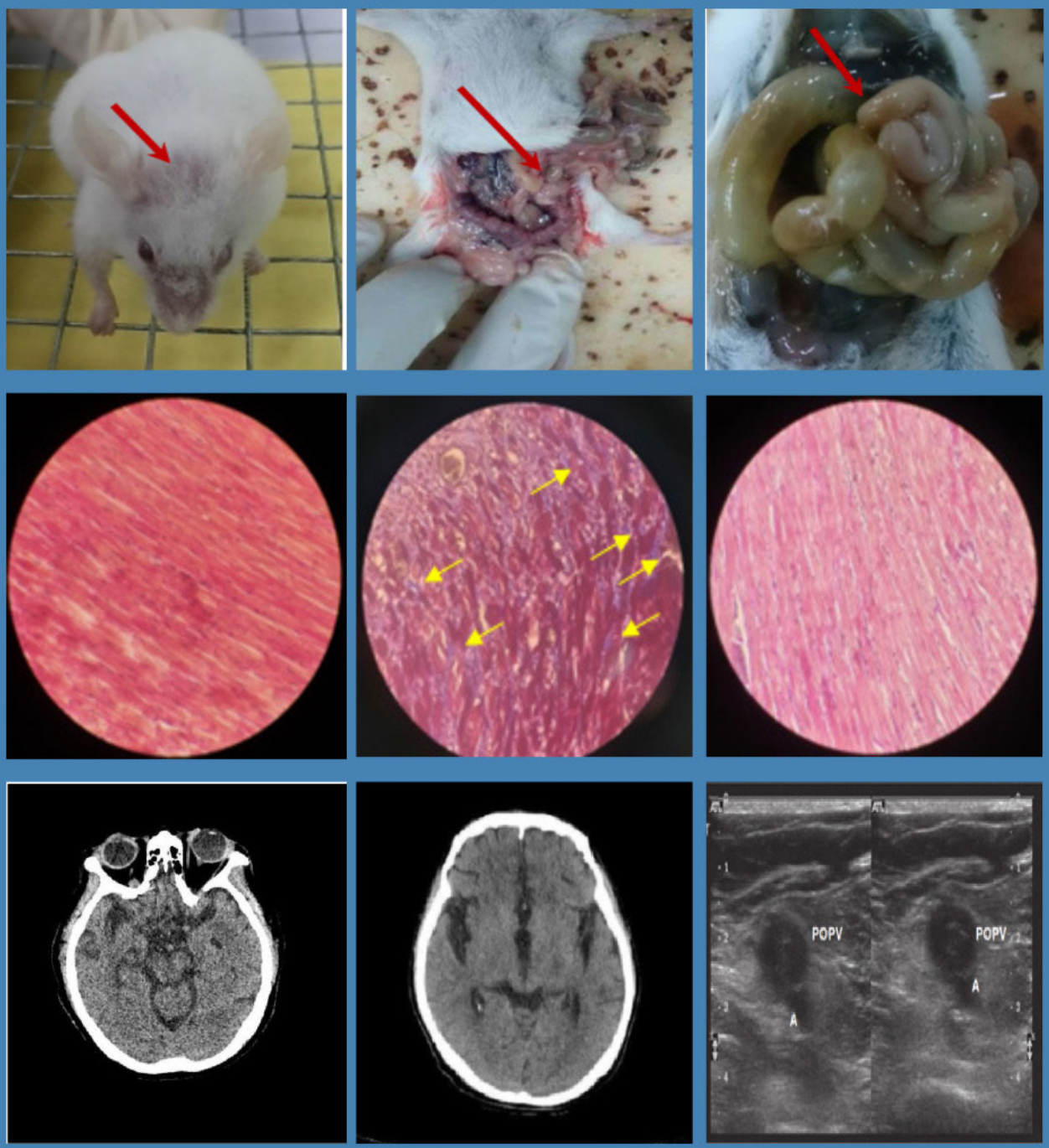

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Serum IL-17 levels correlate with urinary albumin in systemic lupus erythematosus (SLE) pregnant mice model

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ABSTRACT

Women of reproductive age are more likely to have systemic lupus erythematosus (SLE), which frequently results in health issues, particularly during pregnancy. A normal pregnancy's first trimester shows a marked increase in the percentage of Th17 cells, which then steadily declined in the second and third trimesters. Meanwhile, IL-17 level increases in SLE-affected pregnant women. This study aimed to analyze the correlation between serum IL-17 and pregnancy outcome (fetus weight, blood pressure, urinary albumin) in SLE pregnant animal models. Twenty mice were randomly divided into two groups, including the normal pregnant group and SLE-pregnant group. The SLE pregnant mice was made by intraperitoneally induction of 0.5 mL pristane. Serum IL-17 was assayed by enzyme-linked immunosorbent assay (ELISA) method. The serum IL-17 level, the blood pressure and urinary albumin were significantly higher in the SLE pregnant mice group than those of the normal pregnant group ($p< 0.05$). The weight of fetus was significantly smaller in the group of SLE pregnant mice group than the normal pregnant group ($p< 0.05$). There was a significantly positive correlation between the serum IL-17 level and urinary albumin ($p = 0.042$; $r = 0.459$). In conclusion, serum IL-17 levels correlate with urine albumin in SLE pregnant models, but do not correlate with fetus weight and blood pressure.

ABSTRAK

Wanita usia subur cenderung mengalami *systemic lupus erythematosus* (SLE), yang seringkali menimbulkan masalah kesehatan, terutama selama kehamilan. Prosentase sel Th17 meningkat pada trimester pertama kehamilan normal, yang kemudian menurun pada trimester kedua dan ketiga. Sementara itu, kadar IL-17 meningkat pada wanita hamil dengan SLE. Penelitian ini bertujuan untuk mengkaji hubungan antara serum IL-17 dan luaran kehamilan (berat janin, tekanan darah, albumin urin) pada model SLE bunting. Dua puluh mencit secara acak dibagi menjadi dua kelompok, termasuk kelompok bunting dan kelompok bunting dengan SLE. Model SLE pada mencit dibuat dengan induksi 0,5 mL pristan secara intraperitonial. Kadar IL-17 serum ditetapkan dengan *enzyme-linked immunosorbent assay* (ELISA). Kadar IL-17 serum, rerata tekanan darah dan albumin urin lebih tinggi secara nyata pada kelompok tikus SLE bunting dibandingkan kelompok bunting normal ($p<0,05$). Berat janin lebih kecil secara nyata pada kelompok tikus SLE bunting dibandingkan dengan kelompok bunting normal ($p<0,05$). Terdapat hubungan positif yang signifikan antara kadar IL-17 serum dan albumin urin ($p = 0,042$; $r = 0,459$). Simpulan, kadar IL-17 serum berhubungan dengan albumin urin pada model SLE bunting, tetapi tidak berhubungan dengan berat janin dan tekanan darah.

Keywords:
serum IL-17;
systemic lupus erythematosus;
pregnancy outcome;
urine albumin;
animal model

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with multisystemic involvement. It affects practically any organ system and can result in irreversible damage. The etiology of SLE remains incompletely understood and it is believed to be a combination of genetic, hormonal, environmental, and immune factors.[1] This autoimmune disease has a significant female predominance. The onset during reproductive years, coupled with improved survival, has led to increased numbers of pregnancies in women with SLE. The pregnancy outcomes have also significantly improved. The rate of pregnancy loss has decreased from 43% to 17% in recent years.[2] However, SLE patients have smaller children than normal individuals, and SLE pregnancy still carries a high risk of complications.[3-5] The autoreactive adaptive arm (T and B lymphocytes) represents a prominent role in SLE physiopathology, leading to the subsequent production of antinuclear autoantibodies and the consequent deposition of immune complexes throughout the body that can directly induce inflammation.[6] This sustained response causes patients to develop local inflammatory episodes that give rise to a vicious circle in the autoimmune response, leading to a number of immunological abnormalities and tissue destruction.[7] In addition, the involvement of $CD4^+$ T helper cells (Th) in SLE has become increasingly evident.[8] and disturbances in the expression of the Th1/Th2/Th17 cytokine network have also been reported in SLE patients.[8-11]

SLE is more common in women of reproductive age and often causes health problems especially during pregnancy. Pregnant women suffering from SLE have bad complications for the mother and fetus.[12] During pregnancy, when remission SLE before pregnancy less than 6 months, the risk of exacerbation of SLE is 50%.[13] In the first trimester of a normal pregnancy, the percentage of Th17 cells were significantly increased and then gradually decreased in the second and third trimesters. Other studies mention that normal pregnant women had lower number of Th17 cells.[14,15] Meanwhile, pregnant women with SLE contained elevated levels of IL-17.[16] Nonetheless, the pathomechanism of pregnancy in SLE, especially the relationship between the output SLE pregnancies remains unclear. Therefore, this study aimed to analyze the correlation between serum IL-17 with pregnancy outcome in SLE pregnancies models.

MATERIAL AND METHODS

Animals

Female BALB/c mice, 26-28 wk of age, weighing 25–30 g, were used for this study. Twenty mice were randomly divided into two groups with ten mice in each group. The first group was a normal pregnant group as control group. The second group was a SLE-pregnant group (SLE group). Mice were housed in a clean wire cage and maintained under standard laboratory conditions with temperature of 25 ± 3°C and dark/light cycle 12/12 h. Standard diet and water were provided *ad libitum*. Mice were acclimatized to laboratory conditions for one wk before the experiment. Mice care and experimental procedures were approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.

Pristane-induced SLE

SLE model was made according to Satoh *et al.*[17] by an intraperitoneally injection of 0.5 mL pristane (Santa Cruz Biotechnology, USA) at 6-8 wk of age and after 7 d of acclimatization. This SLE model was reported to reproduce

clinical similarities to human SLE and being useful to identify and validate new therapies for the treatment or prevention of this autoimmune disease. After 12 wk of pristane induction, clinical and immunological manifestations experienced by BALB/c mice were observed. The clinical manifestation included hair loss, facial redness, ascites, and intestinal dilatation. While, the immunological manifestation was an increase in antinuclear antibody (ANA). Followed, after 12 wk of pristane induction, mice fertilization was performed.

Breeding

Breeding began after 12 wk of pristane induction. Mice were caged in a constant photoperiod. Virgin female mice were mated with fertile male of the same strain to induce pregnancy. The following morning of finding a vaginal plug was designated as gestational day (GD) 1.[18]

Tissue and blood sampling

Blood samples were taken at day 18 of pregnancy. Surgery and blood collection were carried out at the Pharmacology Laboratory of Universitas Brawijaya, Malang, Indonesia. The sampling stage was started with preparing tools and materials for minor operations such as tweezers, scissors, ether and vacutainers without EDTA. Then the mice were euthanized by anesthetizing with 10% chloroform formalin inhalation to perform surgery to obtain blood. Blood was taken from the heart at approximately 1 mL using a syringe, and then the blood that was drawn was put into a vacutainer tube without anticoagulant and then centrifuged to get serum at 3500 rpm for 15 min. After being centrifuged, the supernatant was taken and transferred to an Eppendorf tube and then stored at -40^{o}C.

Urinary sampling

Urinary albumin measurement was performed once on the 14th day of gestation. Urine samples were obtained the day before the height of urine albumin levels. All the mice were placed in special cages to collect urine for 24 h. Then in the morning, the mice's urine was collected, taken, and stored in a labeled urine bottle. The urine was then transferred into an Eppendorf tube and centrifuged at 3500 rpm for 15 min, and then the urine sample is stored at -20^{o}C.

Analysis of serum IL-17

Analysis of serum IL-17 was performed using IL-17 ELISA kit (Biolegend, USA, Catalog No: 432507). The analysis was conducted according to the detailed instruction in the kit. After blood was taken and put into a blood collection tube without EDTA, blood was centrifuged to obtain supernatant at 35000 rpm for 15 min. Then IL-17 levels were measured by measuring IL-17 cytokine secretion in serum using the ELISA Kit mouse IL-17A method. Overall, the procedure for measuring IL-17A levels was carried out based on standard methods from the Biolegend factory (Biolegend, USA, Catalog Number 432507). Measurement of IL-17A levels began with washing buffer dilution (20x). Aquades was added to 1 mL of wash buffer concentrate, then 2 mL of aquabidest was added to the matrix. One mL of assay buffer was added to standard IL-17 mice. The final standard concentration after dilution was 1000 pg/mL. Standards were prepared using 8 Eppendorf tubes, then standard wells and sample wells were prepared and then washed using wash buffer 4 times.

Fifty µL of each matrix were added to the standard wells, and 50 µL of serum samples were put to the sample wells. The wells were then rotated to ensure that the solution was homogeneous. Then,

the wells were incubated at 37^{0}C for 120 min. After incubation, the wells were washed four times with wash buffer, and 100 µL mouse IL-17A detection antibody was added to all wells (standard and sample). Then the wells were rotated so that the solution was homogeneous. The wells were incubated again at 37^{0}C for 60 min, and then the wells were washed four times with a wash buffer. 100 µL of HRP A was added to each well, and then the wells were rotated so that the solution was homogeneous, then incubated again at 37^{0}C for 30 min. The wells were washed four times with wash buffer. After washing, 100 µL of substrate solution was added to each well. The color changed to blue (in the darkroom). Incubation were added for another 30 min, then 100 µL stop solution was added to the well. the color changed to yellow. The results were read on the Elisa Reader Microplate machine at λ 450 nm.

Measurement of mean arterial blood pressure

Blood pressure was measured using a Kent Scientific CODA. Blood pressure measurement was performed by calculating a mean arterial blood pressure (MAP) of the pregnant SLE group and then compared to the average of the normal pregnant group.

Analysis of urinary albumin

Analysis of urinary albumin was performed using ELISA kit (Elabscience, Catalog No; E-EL-M0656). According to the kit's comprehensive instructions, the analysis was conducted on 12 h urine samples. The centrifuged urine sample was stored at –20^{0}C and removed from the refrigerator, waiting for it to thaw. The required urine sample was 100 µL. The ELISA kit was removed from the refrigerator 30 min before use, and standard wells and samples were prepared. Wash buffer (25x) was prepared by adding 24 mL of distilled water with 1 mL of concentrated wash buffer. The standard was prepared, and 1 mL of diluent and reference samples were added and then allowed to stand for 10 min (after the standard was diluted, it would be 1000 pg/mL). The standard was made using 8 Eppendorf tubes, and then 100 µL standard was put into the well and a 100 µL urine sample was added. After that, the well containing the standard solution and the urine was rotated for 5 min to homogenize the solution.

The wells were incubated at 37 ^{0}C for 90 min. Then the wells were washed with wash buffer 5x. Each well received 50 µL of Biotin Ab solution, which was subsequently homogenized by rotating the wells. The process was followed with incubation at 37^{0}C for 60 min. The next stage was incubated at 37^{0}C for 60 min. After incubation, the wells were washed with wash buffer 3x. Fifty µL HRP conjugate was added to each well. Then the wells were rotated so that the solution was homogeneous. The wells were incubated again at 37^{0}C for 30 min. Then the wells were washed five times with wash buffer. After washing, 90 µL of substrate solution was added to each well, and the color changed to blue (in the darkroom). Incubate for 15 min, then stop solution is added to the well, and the color changes to yellow. The results are read on the ELISA reader machine. Urinary albumin levels with a unit value of µg/mL were recorded and analyzed from the results obtained.

Measurement of fetal weight

Fetal weight was measured using analytical scales METTLER AE50.

Ethics

This study was approved by the Health Research Ethics Committee of the Faculty of Medicine Brawijaya University.

Statistical analysis

All variables met the parametric prerequisite test. All data proved to be customarily distributed then data analysis was continued with a correlation test to determine the relationship between IL-17 serum levels and pregnancy outcomes (urine albumin levels, blood pressure, and fetal weight) using the Pearson correlation test.

RESULTS

The clinical manifestations in pristane-induced SLE mice model

Clinical manifestations in pristane-induced SLE mice model are presented in FIGURE 1. Among the ten pristane-induce SLE mice model, 5 (50%) mice experienced ruffled feathers, 2 (20%) mice had facial flushing, 1 (10%) mice had ascites (10%), 1 (10%) mice had gut dilatation and 1 (10%) mice had no clinical manifestations of SLE (TABLE 1).

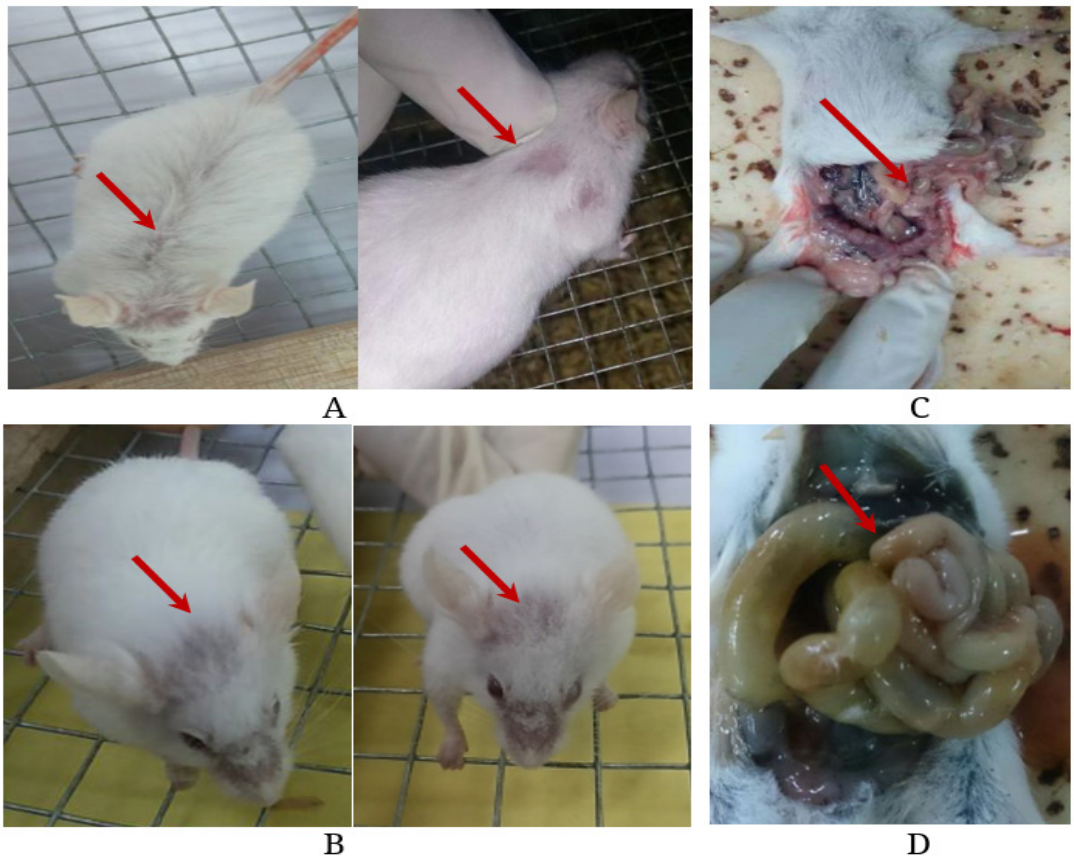

FIGURE 1. Clinical manifestation of SLE mice, including ruffled feathers (A), facial flushing (B), ascites (C), and gut dilatation (D).

The IL-17 serum levels

Serum IL-17 levels were significantly higher (p=0.042) in the SLE pregnant mice group (1076 ± 11.4 ng/dL) the normal pregnant mice group (1006 ± 101.3 ng/dL) (FIGURE 2).

The outcome of pregnancy

Pregnancy outcome of normal pregnant mice and SLE pregnant mice group including the arterial pressure, urinary albumin, and fetal weight are presented in TABLE 2. The blood pressure of the SLE pregnant mice group (91.8 ± 22.32 mmHg) were significantly higher than those the normal pregnant group (69.8 ± 7.62 mmHg) (p = 0.013). Urine albumin levels of the SLE pregnant mice group (1402.3 ± 401.5 ng/mL) were also significantly higher than those the normal pregnant group (132.3 ± 197.9 ng/mL) (p= 0.000). Whereas, fetal body weight of the SLE pregnant mice group (0.8 ± 0.2 g) were significantly lower than those the normal pregnant group (1.1 ± 0.1 g) (p= 0.000).

The correlation of biomarkers with pregnancy outcome

TABLE 3 presents the correlation of serum IL-17 with pregnancy outcome. The levels of serum IL-17 was significantly correlated with urinary albumin levels (p = 0.042; r = 0.459). There was no significant correlation between serum IL-17 with mean blood pressure and fetal body weight.

TABLE 1. The distribution of clinical manifestations in pristane-induced SLE mice model

The clinical manifestations	Pristane-induced SLE mice [n (%)]
Ruffled feathers	5 (50)
Facial flushing	2 (20)
Ascites	1 (10)
Gut dilatation	1 (10)
No clinical manifestation	1 (10)

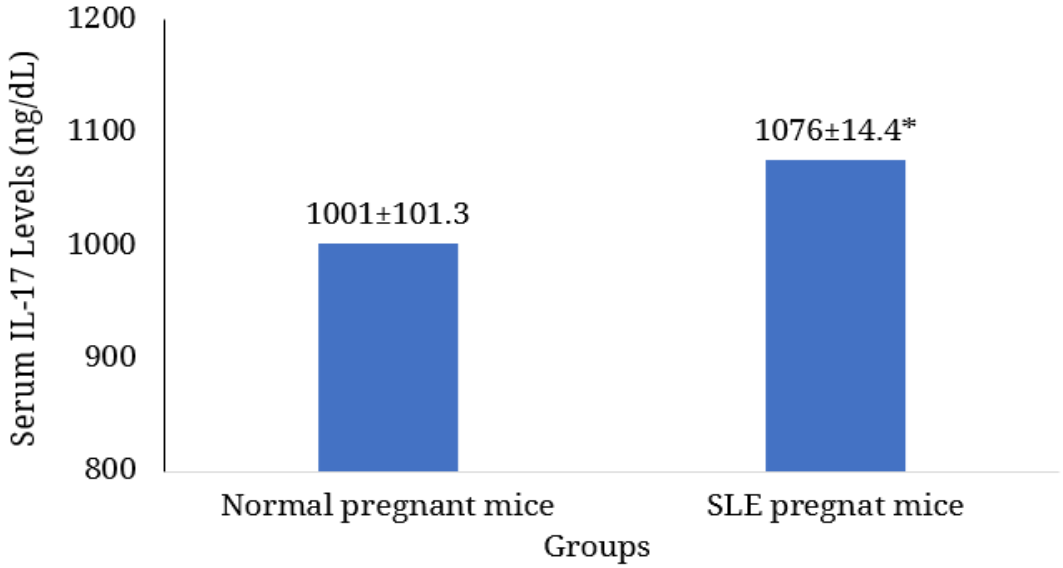

FIGURE 2. Serum IL-17 level in the normal pregnant and the SLE pregnant mice groups (*significant different p<0.05.)

TABLE 2. The outcome of pregnancy in each group

Clinical Characteristic	Normal pregnant (n=10)	SLE pregnant (n=10)	p
Blood pressure (mmHg)	69.80 ± 7.62	91.8 ± 22.32	0.013
Urine albumin level (ng/mL)	132.3 ± 197.9	1402.3 ± 401.5	0.000
Fetal body weight (g)	1.1 ± 0.1	0.8 ± 0.2	0.004

TABLE 3. The correlation between biomarkers and pregnancy outcomes

Variable	Urinary albumin	Blood pressure	Fetal body weight
IL-17	p=0.042*; r=0.459	p=0.172; r=0.318	p=0.894; r=-0.032

*significant with p<0.05

DISCUSSION

Systemic lupus erythematosus (SLE) is a potentially severe autoimmune disease characterized by increased titers of serum autoantibodies.[19] Common autoantibody-mediated damage mechanisms in SLE include immune complex-mediated damage, cell surface binding, and cytotoxicity, reactivity with autoantigens expressed on apoptotic or activated cell surface, penetration into living cells, binding to cross reactive extracellular molecules.[20] In this study, the levels of serum IL-17 of the SLE pregnant mice group were significantly higher than those the normal pregnant group ($p < 0.05$). A previous study reported that injection of pristane could overstimulate immune

response reaching auto reactive level.[21] SLE is associated with impaired disposal of apoptotic cell products due to deficiencies of scavenging molecules in phagocytic cells, such as scavenger receptors, or complement components such as C1q which facilitate phagocytosis of apoptotic cells. These apoptotic cells can induce and augment Th17 and the doubly potent Th1/Th17 responses.[22,23] In patients with SLE, there is an increased percentage of Th17 cells and its activity which is accompanied by the rising of the cytokine IL-17.[24,25] In this study, there is a significant positive correlation between the levels of serum IL-17 with urinary albumin ($p = 0.042$; $r = 0.459$). This finding indicated that serum IL-17 might be correlated with the changes in glomerular barrier alteration. Our finding was in line with previous studies that IL-17 might be correlated with the level of disease activity on the SLE.[26,27] An increase in the activity of Th17 cells can induce the production of inflammatory mediators and toxic to the tissue.[28,29]

CONCLUSION

In conclusion, there is a correlation between serum IL-17 levels with urinary albumin in pregnant SLE mice models. However, there is no correlation between serum IL-17 levels with fetus weight and blood pressure.

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Protective effect of corncob extract cream on guinea pig (*Cavia porcellus* sp) skin pigmentation exposed to ultraviolet B (UVB) rays

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ABSTRACT

Ultraviolet B (UVB) rays exposure causes skin inflammation and pigmentation lead to decrease skin lightness. Corncobs (*Zea mays*) contain flavonoids which can act as antioxidant to prevent free radicals and protect the skin pigmentation. This study aimed to evaluate the protective effect of corncob extract cream on skin pigmentation exposed to UVB rays. This pre-posttest control group study was applied to 25 guinea pigs (*Cavia porcellus* sp) randomly divided into five groups. Corncob extract cream was given every day 20 min before and 4 h after UVB exposure. The UVB exposure total dose was 780 mJ/cm^2. Mexameter examination was carried out on the 1st day and 28th day after treatment. There were significantly differences in the lightness level and the mean melanin index (MI) difference of guinea pigs before and after intervention on various groups ($p<0.05$). No significantly different of the MI between pre- and post-treatment was observed on normal control group (0.22) and negative control group (-1.06) ($p>0.05$). However, significantly different of the MI was observed on positive control group (-4.01), corncob 40% group (-2.72), and corncob 30% group (-2.03) ($p<0.05$). In conclusion, corncob extract cream can inhibit the skin pigmentation due UVB rays exposure.

ABSTRAK

Paparan sinar ultraviolet B (UVB) menyebabkan inflamasi dan pigmentasi kulit sehingga kecerahan kulit berkurang. Tongkol jagung (*Zea mays*) mengandung flavonoid yang dapat berperan sebagai antioksidan untuk mencegah radikal bebas dan melindungi pigmentasi kulit. Penelitian ini bertujuan untuk mengkaji efek protektif krim ekstrak tongkol jagung terhadap pigmentasi kulit akibat paparan sinar UVB. Penelitian dengan rancangan kelompok kontrol *pre-posttest* ini dilakukan pada 25 ekor marmut (*Cavia porcellus* sp) yang dibagi secara acak menjadi 5 kelompok. Krim ekstrak tongkol jagung diberikan setiap hari 20 menit sebelum dan 4 jam setelah paparan sinar UVB. Dosis total paparan UVB adalah 780 mJ/cm2. Pemeriksaan dengan mexameter dilakukan pada hari ke-1 dan hari ke-28 setelah perlakuan. Terdapat perbedaan bermakna tingkat kecerahan dan perbedaan rerata indeks melanin (MI) marmut antar kelompok sebelum dan sesudah intervensi ($p<0,05$). Tidak ada perbedaan signifikan MI antara sebelum dan sesudah pengobatan pada kelompok kontrol normal (0,22) dan kelompok kontrol negatif (-1,06) ($p>0,05$). Namun terdapat perbedaan signifikan MI pada kelompok kontrol positif (-4,01), kelompok tongkol jagung 40% (-2,72), dan kelompok tongkol jagung 30% (-2,03) ($p<0,05$). Simpulan, krim ekstrak tongkol jagung dapat menghambat pigmentasi kulit akibat paparan sinar UVB.

Keywords:
skin lightness;
corn cobs;
UVB rays;
melanin index;
in vivo

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INTRODUCTION

Most Asian women emphasize skin brightness because they believe bright skin is light.[1] Melanin, the pigment responsible for skin lightness, protects skin and hair cells from ultraviolet (UV) exposure.[2] Lightening products are commonly used to obtain lighter skin. Hence, the widely circulating skin-lightening creams or drugs in markets.[1] Throughout 2018, the National Agency of Drug and Food Control (NADFC) of Republic of Indonesia found 230 cosmetic products containing mercury.[3] Mercury is highly toxic to organs. In low doses, it causes allergy, irritation, and black spots, while in high doses, it could damage the renal, neurons, and brain.[4] Hydroquinone is a skin-lightening agent that inhibits melanogenesis. However, it potentially causes significantly ochronosis in long-term use in the dosage exceeds 2%.[5]

Corn (*Zea mays*) is widely cultivated in Indonesia. The fruit of this plant has a part called a corncob, which keeps the nutrition for corn seed growth. It is estimated at around 40-50% of total corn weight. To date, corncob waste is rarely used.[6] However, previous studies have reported that corncob extract possesses phytochemicals and a phenolic compound having potency as a singlet oxygen quencher and active sunscreen compound.[7,8]

Additionally, phenol and flavonoid were discovered to possess antioxidant activity.[9] A previous study about the antioxidant activity of corncob revealed that corncob extract with a concentration of 40% has a phenolic concentration of 81.53 mg/kg. Therefore, it has a free radical blocking activity.[10] As an antioxidant, flavonoids can neutralize free radicals (reactive oxygen species/ROS), which give electrons or hydrogen, causing a stable non-radical molecule.[10]

Guo *et al*.[9] reported that corncob and corn hair extracts comprise quercetin compounds that could ward free radicals off. One of the free radical sources is UV rays which causes skin changes such as pigmentation. The corn cob can be developed as a melanin pigment production inhibitor due to its antioxidant activity in cream and ability to increase the lightening skin effect. This study aimed to investigate the effect of 30% corncob extract and 40% on skin lightening of guinea pigs exposed to UVB.

METHODS

Animal and experimental design

This was an experimental pretest-posttest control group study employed the guinea pigs were distributed into five groups using a simple random sampling technique. The number of guinea pigs (*Cavia porcellus* sp.) was 5 in each group based on the Federer sample size formula. A total of 25 guinea pigs were used in the study. All the guinea pigs were given 7 days for adaptation, including feeding and standard treatment before being enrolled in the experiment. The guinea pigs were divided into five groups namely the control normal group (G1) or the group without treatment, the negative control group (G2) or group was given basic cream, the positive control group (G3) was given hydroquinone cream, G4 group, the one who was given 30% corncob extract cream, and the G5 group, the one who was given 40% corncob extract cream.

Examination of the skin lightness

The cream was administered each day for 20 min before UVB exposure and 4 h after UVB exposure. To all groups, after hair removal, the dorsal skin of the guinea pigs was exposed to UVB radiation, with total UVB exposure was 780 mJ/cm^2 for 28 d.[11]

Lightness was measured by sticking the Mexameter probe to the guinea

pig's shaved skins until the melanin index (MI) was displayed on day one before the application of the cream (pre-treatment) and day 28 after the application (post-treatment). This probe ejects radiation that will be reflected by skin tissue and received by the probe's receptor. Radiation caught was interpreted as a MI (0-999 scale). This study was approved by the Medical Bioethics Research Committee, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang (ref no: 383/XI/2020/ Komisi Bioetik on November 30th, 2020).

Statistical analysis

Data were presented as MI and analyzed using SPSS version 20. Normal distribution and homogenous data were analyzed using Shapiro Wilk test and Lavene's test. Normally distributed data were then analyzed using one-way Anova, continued by pair t test to evaluate the difference between pre- (1st day) and post-treatment (28th day) in each group.

RESULT

The MI on pre- (1st day) and post-treatment (28th day) is presented in TABLE 1. The lowest MI were observed on negative control (G2) both on pre- (222.39±19.18) and post-treatment (221.33±8.40). The highest MI were observed on positive control (G3) both on pre- (589.65±63.92) and post-treatment (585.64±64.21). Furthermore, no significantly different of the MI between pre- and post-treatment was observed on normal control group (0.22) and negative control group (-1.06) ($p>0.05$). Whereas, significantly different of the MI between pre- and post-treatment was observed on positive control group (-4.01), Corncob 40% group (-2.72), and Corncob 30% group (-2.03) ($p<0.05$).

The highest MI reduction after post-treatment compared to pre-treatment was observed on positive control group (-4.01), followed by Corncob 40% group (-2.72), and Corncob 30% group (-2.03) (FIGURE 1).

TABLE 1. Melanin index on 1st d and 28th day in each group

Group	MI on 1st day	MI on 28th day	Mean difference
Normal control (G1)	239.28±8.66	239.50±8.40	0.22
Negative control (G2)	222.39±19.18	221.33±8.40	-1.06
Positive control (G3)	589.65±63.92	585.64±64.21	-4.01*
Corncob 30% (G4)	345.48±72.75	343.48±73.58	-2.03*
Corncob 40% (G5)	461.11±63.25	458.39±61.13	-2.72*

*significantly different (paired t test $p<0.05$)

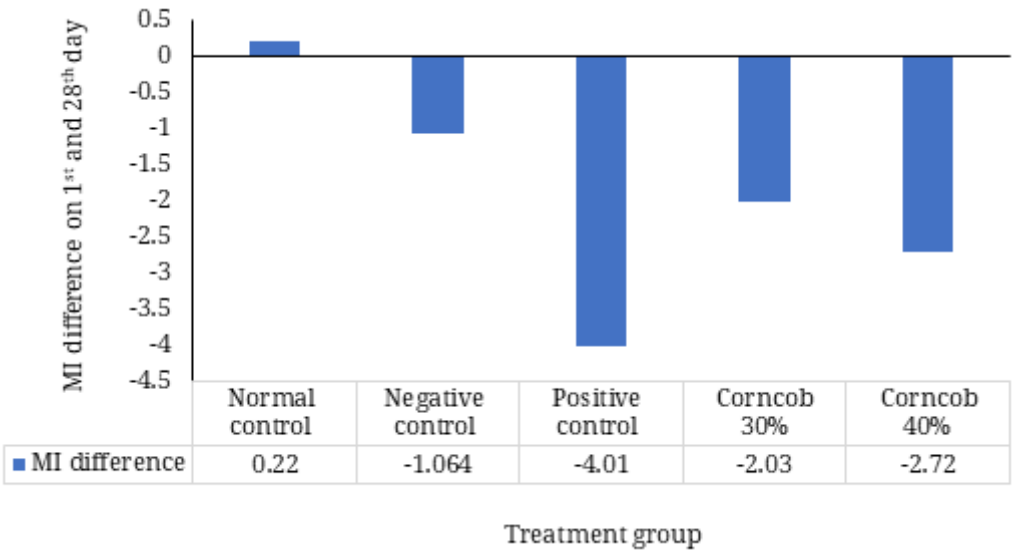

FIGURE 2. Melanin index mean difference between 1st and 28th d in all groups

DISCUSSION

UVB-induced ROS can induce cytokine secretion, stimulating melanocytes to produce excess melanin pigment which be transported to keratinocytes in all epidermal skin layers and cause black skin color or skin pigmentation.[12,13] Photoprotection and sunscreens protective against both UV and visible light are recommended by medical doctor. A lot of preparations originally from conventional drugs, herbal medicine, and cosmetic products are available in the market to protect the skin pigmentation. Skin melanin index is often used to evaluate the effectiveness of the photoprotection of a preparation.

The mean MI after post-treatment compared to pre-treatment on positive control group (-4.01), corncob 40% group (-2.72), and corncob 30% group (-2.03) significantly reduced ($p<0.05$). Although the MI difference after administration of the corncob 40% and 30% creams were lower than that after administration of hydroquinone creams (positive control), however they were higher than that after administration of the basic cream (negative control). It was indicated that the administration of the corncob creams can inhibit melanin pigment production due to UV light exposure.

Hydroquinone is the most frequently used photoprotection or skin lightening. Hydroquinone is a strong tyrosinase inhibitor, currently known as the gold standard for hyperpigmentation therapy.[14,15] Hydroquinone, a depigmenting compound, prevents dihydroxyphenylalanine conversion into melanin by inhibiting the tyrosinase enzyme as a competitive inhibitor.[15,16] The IC_{50} values for hydroquinone in the mushroom tyrosinase inhibition cover a wide range from 1.113 to 680 μmol/L.[14] The lowest IC_{50} value indicates a very strong potential as a tyrosinase inhibitor compared to the anti-tyrosinase potential of other compounds. However, the long use of hydroquinone with dosage over 2% may cause side effects such as irritation, rebound phenomenon, and ochronosis.[5]

Corncob (*Zea mays*) acts as food storage, supplying corn seed growth as long as it is attached to the cob and has active substances that can potentially be used as active antioxidant compounds.[17,18] It contains active phenolic substances in the form of flavonoid. Flavonoid is used as a potential phytochemical antioxidant that can fend off free radicals.[10] Flavonoid has a good affinity with tyrosinase enzymes and prevents dopachrome and melanin formation lead to inhibit skin pigmentation.[19] Quercetin in corncob extract and corn hair can deflect free radicals. One of the free radical sources is UV light which can cause changes in the skin, including pigmentation.[9]

Some herbal preparations have been studied to evaluate their skin ligthness activity. Trifena *et al.*[20] reported *in vitro* antioxidant activity and *in vivo* skin lightness effectiviy on the momen volunteers of a cream containing combination of mangosteen peel extract (*Garcia mangostana* L.) and gotu kola herbs extract (*Centella asiatica* L.). Furthermore, Sesamol, an active component in sesame seeds, has been reported as a potent depigmenting agent in the animal model. Sesamol was proven reduce UVB-induced tyrosinase, TRP-1, TRP-2, and MITF expression in the epidermis of the skin.[21]

Some limitations were identified in this study. The guinea pigs used have a variety of skin colors which could affect preliminary study results. Furthermore, only two dosage variations of corncob extract were used in this study. These dosages are lower than the commonly used dose in a lightening cream. Therefore, further study with higher dosage is needed in order to find a dose that equivalent with hydroquinone. Quercetin levels as chemical marker in corncob extract was not also determined in this study.

CONCLUSION

In conclusion, corncob (*Z. mays*) extract cream can inhibit the skin pigmentation of guinea pig exposed to UVB rays. Although, its activity at 40% extract dose is lower than hydroquinone, however it is higher than the basic cream. A further study with higher extract dose is needed to obtain effect dose that similar with hydroquinone. Specific standardization of the extract using chemical marker is also needed.

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Protective effect of *Moringa oleifera* leaves extract on cardiac fibrosis of streptozotocin-induced diabetic rats

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disease characterized by chronic hyperglycemia that induces excessive reactive oxygen species (ROS) production and causes oxidative stress. Diabetic cardiomyopathy is a diabetic complication characterized by structural and functional changes of the myocardium. Fibrosis is one of the pathological features of diabetic cardiomyopathy. *Moringa oleifera* leaves have been reported to possess antidiabetic and antioxidant activities which could prevent diabetic complications such as cardiomyopathy. A previous study reported that *M. oleifera* leaves extract have protective effects to the kidneys and liver of rats exposed to oxidative stress. This study aimed to investigate the protective effect of the *M. oleifera* leaves extract on cardiac fibrosis of rats induced by streptozotocin (STZ). This was an experimental study using a posttest-only control group design. Thirty-three male Wistar rats were randomly divided into three groups i.e. normal control group (Group 1) were administered normal saline, diabetic control group (Group 2) were administered normal saline, and diabetic treatment group (Group 3) were administered *M. oleifera* leaves extract. Diabetes induction of rats was conducted by intraperitoneally injection of STZ at dose of 45 mg/kg BW. The *M. oleifera* leaves extract at a dose of 1000 mg/kg BW was administered orally one time a day for 28 days. Statistical analysis was performed using the Kruskal-Wallis test followed by Mann Whitney. A significant difference in cardiac fibrosis occurrence between three groups was observed ($p < 0.05$). No cardiac fibrosis was observed in normal control group, In figure 3 we explained that fibrosis was observed in 8 rats of diabetic control group. Only 2 rats in the treatment group (G3) had cardiac fibrosis. In conclusion, *M. oleifera* leaves extract can inhibit cardiac fibrosis in STZ-induced diabetic rats.

ABSTRAK

Diabetes melitus (DM) merupakan penyakit metabolik yang ditandai dengan hiperglikemia kronis yang menyebabkan produksi spesies oksigen reaktif (ROS) berlebihan dan menyebabkan stres oksidatif. Kardiomiopati diabetik adalah komplikasi diabetes yang ditandai dengan perubahan struktural dan fungsional miokardium. Fibrosis adalah salah satu gambaran patologis kardiomiopati diabetik. Daun kelor (*M. oleifera*) dilaporkan memiliki aktivitas antidiabetik dan antioksidan yang dapat mencegah komplikasi diabetes seperti kardiomiopati. Penelitian sebelumnya melaporkan bahwa ekstrak daun kelor memiliki efek protektif terhadap ginjal dan hati tikus yang terpapar stres oksidatif. Penelitian ini bertujuan untuk mengetahui efek protektif ekstrak daun kelor terhadap fibrosis jantung tikus yang diinduksi streptozotocin (STZ). Penelitian ini merupakan penelitian eksperimental dengan menggunakan rancangan *posttest-only control group*. Tiga puluh tiga ekor tikus Wistar jantan dibagi secara acak menjadi tiga kelompok yaitu kelompok kontrol normal (K1) yang disuntik dan diberikan larutan garam normal, kelompok kontrol diabetes (K2) diberikan larutan garam normal, dan kelompok perlakuan diabetes (Kelompok 3) diberikan ekstrak daun kelor. Induksi diabetes pada tikus dilakukan dengan cara penyuntikan STZ secara intraperitoneal dengan dosis 45 mg/kg BB. Ekstrak daun kelor dosis 1000 mg/kg BB diberikan secara oral satu kali sehari selama 28 hari. Analisis statistik dilakukan dengan menggunakan uji Kruskal-Wallis yang diikuti oleh Mann Whitney. Terdapat perbedaan yang signifikan dalam kejadian fibrosis jantung antara tiga kelompok yang diamati ($p < 0,05$). Tidak ada fibrosis jantung yang diamati pada kelompok kontrol normal (K1), sedangkan fibrosis jantung diamati pada semua tikus (9 tikus) dari kelompok kontrol diabetes (K2). Hanya 2 tikus pada kelompok perlakuan (K3) yang mengalami fibrosis jantung. Kesimpulannya, ekstrak daun kelor dapat menghambat fibrosis jantung pada tikus diabetes yang diinduksi STZ.

Keywords:
diabetes;
oxidative stress;
cardiac fibrosis;
antioxidant;
Moringa oleifera

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1] According to International Diabetes Foundation (IDF) in 2019, DM in Indonesia ranked seventh in the world with 10.7 million cases.[2] Uncontrolled DM can cause complications affecting the heart, kidneys, nerves and blood vessels.[3] Diabetic cardiomyopathy is a diabetic complication characterized by structural and functional changes of the myocardium, one of them is cardiac fibrosis.[4] The cardiac fibrosis can increase the risk of ventricular stiffness, which impairs cardiac contractility.[5]

According to a cohort study conducted over 5.5 years in 1.9 million patients with type 2 diabetes mellitus (T2DM), heart failure is the second most common manifestation of cardiovascular disease after peripheral arterial disease.[6] Another study reported that 32.3% of patients with T2DM experienced heart failure in less than 5 yr, and 67.7% of patients after 5 yr or more.[7] The cardiomyopathy also cause impairment of cardiac function which characterized by diastolic dysfunction. It was reported that the prevalence of diastolic dysfunction in patients with T2DM is between 40-60%.[8]

Hyperglycemia induces excessive production of reactive oxygen species (ROS) which further caused oxidative stress.[9] Oxidative stress has an important role in the pathogenesis of cardiac fibrosis through activation of the transforming growth factor (TGF)-β leads to the activation of the suppressor of mothers against decapentaplegic (SMAD) signaling pathway included SMAD-2/3.[10] In the nucleus, the SMAD-2/3 complexes regulate the transcription of target profibrotic genes encoding proteins involved in extracellular matrix production, including collagen.[11] Streptozotocin (STZ) is a diabetogenic agent that widely used in experimental studies.[12] Histopathological evaluation of the cardiac tissue of diabetic animal induced by STZ showed that diabetes causes structural changes in myocardium. These structural changes are caused by the excessive production of ROS in the myocardium due to hyperglycemic conditions.[13] A previous study also reported that diabetic rats induced by STZ show an increase in the collagen deposition.[14]

Moringa oleifera leaves have been reported to possess antidiabetic and antioxidant activities, which are helpful for the treatment of diabetes and its complications.[15] Quercetin in *M. oleifera* leaves extract was reported as one of the active constituents that is responsible for its high antioxidant activity.[16] *Moringa oleifera* exhibits cardioprotective effects in cardiac damage and vascular dysfunction due to its antioxidant activity.[17] In addition, the rich polyphenolic content of *M. oleifera* reduces the myocardial damage and decreases the oxidative stress.[18] Previous studies reported that administration of *M. oleifera* leaves extract at a dose of 1000 mg/kg BW exhibits protective effects to the kidneys and liver of rats exposed to oxidative stress.[19,20] The aim of this study was to investigate the protective effect of the *M. oleifera* leaves extract on cardiac fibrosis of rats induced by STZ.

MATERIAL AND METHODS

M. oleifera leaves extract preparations

The leaves of *M. oleifera* were obtained from a farmer in Wuluhan District, Jember Regency, and were authenticated at the Herbal Materia Medica Laboratory, Batu, East Java. The leaves were then washed and dried in oven at a temperature of 55-60°C. Once dried, the leaves were ground into a fine powder using a blender and

sieve. The dried and powdered leaves were extracted by maceration using 96% ethanol for 72 h. The mixture was stirred using an orbital shaker to facilitate the extraction process. After 72 h of maceration, the mixture was filtered using Whatman filter paper. The filtrate was then evaporated using water bath at a temperature of 70 ^{o}C. The dried extract obtained was then kept in a refrigerator until used.

Animals and design

This was an experimental laboratory study using a posttest-only control group design. Thirty-three male Wistar rats with weighing 200-300 g at 8-12 wk of age were randomly divided into three groups i.e. normal control rats' group (Group 1) were injected and administered normal saline, diabetic control rats' group (Group 2) were administered normal saline, and diabetic treatment rats' group (Group 3) were administered *M. oleifera* leaves extract. The rats were housed in individual cages, which were cleaned every 2-3 d in a wk. Rats were given standard feed and water *ad libitum*. The *M. oleifera* leaves extract at dose of 1000 mg/BW was orally administered daily started from day 4 and continued for the next 28 d. The rats' body weight was measured five times during the study using a digital scale. This study has been approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, University of Jember, with ref:1599/H25.1.11/KE/2022.

Induction of diabetes by streptozotocin

Diabetes induction was performed by a single intraperitoneal injection of freshly STZ at a dose of 45 mg/kg BW by dissolved in 0.1 M of cold citrate buffer at pH 4.5 to overnight fasted male Wistar rats. After STZ induction, the rats were provided with 10% dextrose overnight to prevent hypoglycemic shock. Three days after STZ induction, the fasting blood glucose levels were measured using EasyTouch glucometer. Rats with fasting blood glucose level (BGL) of greater than 200 mg/dL were considered as diabetic rats and included in this study.

Histological examination

The histopathological examination of was conducted at the Biomedical Laboratory, Faculty of Dentistry, University of Jember. Rats were sacrificed by intraperitoneal injection of pentobarbital at dose of 150 mg/kg BW after 4 wk of treatment. The heart were cleaned with 0.9% NaCl solution to remove any remaining blood or dirt and were preserved in a jar containing a 10% buffer neutral formalin solution.[21] Masson's trichrome staining was used to identify collagen deposition in the cardiac tissue. Each preparation was examined under a microscope at magnification ranging from 100x to 400x and at five fields of view to evaluate the collagen deposition in cardiac tissue. Collagen deposition was stained blue, whereas the cardiac muscle was stained red.[22] The histopathological examination was carried out by two individuals using a single blind technique under the supervision of an anatomical pathologist.

Statistical analysis

Statistical analysis was performed using SPSS version 23 software. Statistical differences were assessed using the Kruskal-Wallis test, followed by Mann Whitney Test, due to not normal data distribution. Differences between groups were considered significant if a p value of <0.05.

RESULTS

Streptozotocin induction

The BGL of normal control group

(G1), diabetic control group (G2) and treatment group (G3) are presented in TABLE 1. The mean BGL of G1 is 108.91 ± 9.101 mg/dL (<200 mg/dL) which considered as normal rats. The mean BGL of G2 and G3 are 437.82 ± 38.126 mg/dL; 424.91 ± 41.469 mg/dL (>200 mg/dL) which considered as diabetic rats (TABLE 1).

Body weight of rats after STZ induction

The mean growth of rats body weight in all groups are presented in FIGURE 1. No significant difference in the body weight of rats in all groups in the week 0 ($p>0.05$). A significant difference in the body weight of rats between normal control group (G1) with diabetic control group (G2) and treatment group (G3) was observed ($p<0.05$). The growth of rats body weight was only observed in normal control group (G1), whereas in diabetic control group (G2) and treatment group (G3), the loss of rats body weight during study in the week 1 to 4 was observed.

Protective effect of *M. oleifera* leaves extract

Among 11 rats induced by STZ, only 9 rats in each group were survive and eligible for histopathological examination. Histopathological features of cardiac tissues of rats in each group are presented in FIGURE 2 and the results of histopathological examinations are presented in FIGURE 3. Significantly different in cardiac fibrosis between the three groups was observed ($p<0.05$) No fibrosis was observed in normal control group (G1), In figure 3 we explained that fibrosis was observed in 8 rats of diabetic control group and in 2 rats of treatment group (G3).

TABLE 1. Rats fasting BGL (mg/dL) in all of groups after STZ induction

Group	n	Mean ± SEM	Min	Max
Normal control group (G1)	11	108.91 ± 9.101	66	156
Diabetic control group (G2)	11	437.82 ± 38.126	248	600
Treatment group (G3)	11	424.91 ± 41.469	208	600

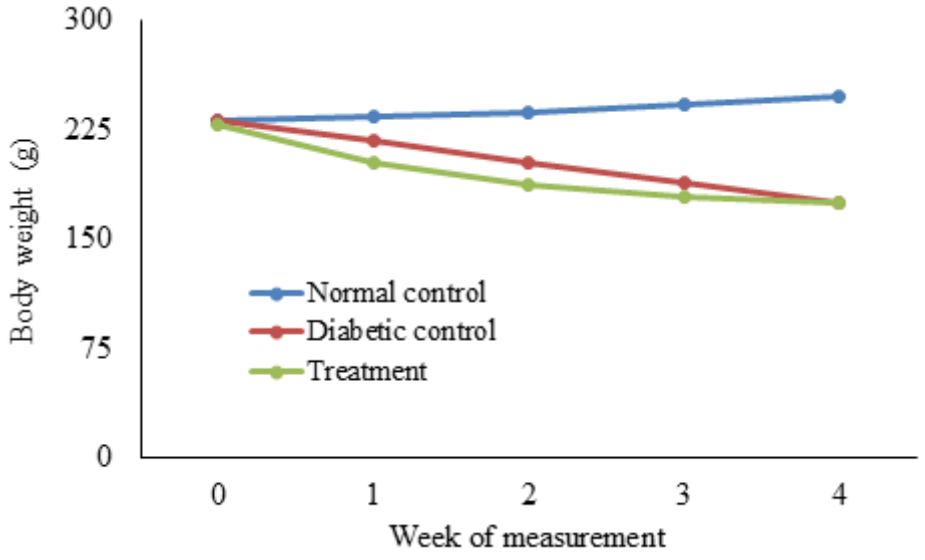
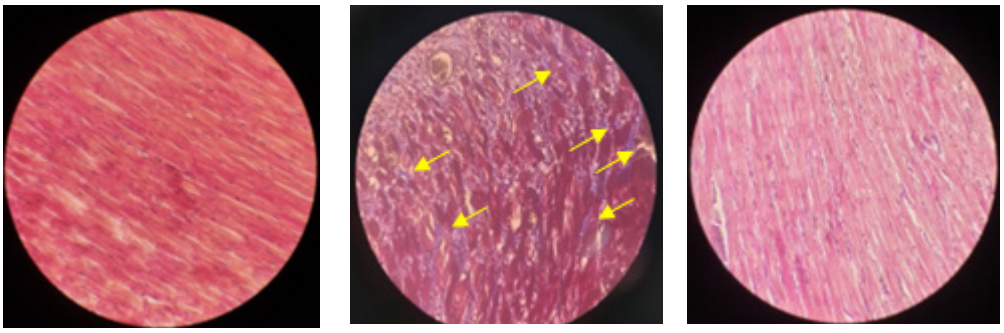


FIGURE 1. Mean body weight of rats



A. Normal control B. Diabetic control C. Treatment

FIGURE 2. Histopathological features of cardiac tissues of rats in each group after microscopic examination with 400x magnification. A. Normal control: no fibrosis was observed; B. Diabetic control: fibrosis was observed (yellow arrow); C. Treatment: no fibrosis was observed.

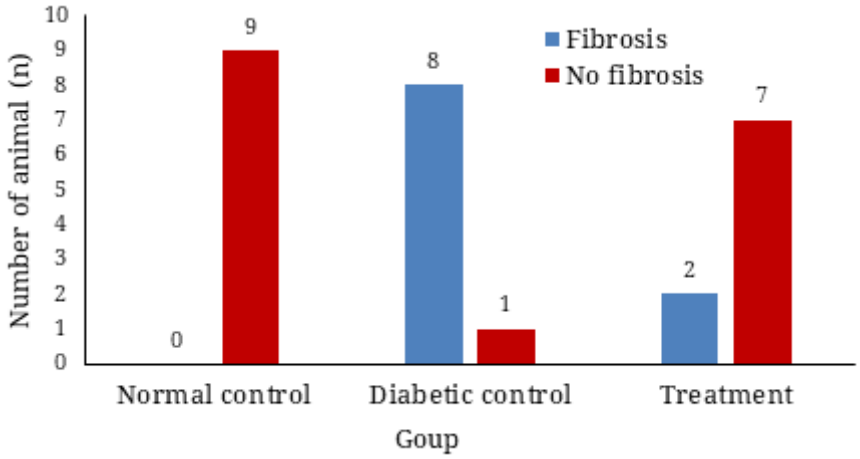


FIGURE 3. The results of histopathological examination of cardiac tissues of rats in each group.

DISCUSSION

In this study STZ is used to induce diabetes of rats. Streptozotocin-induced diabetes model is widely used in various studies related to diabetes in animal model. Streptozotocin is a naturally occurring chemical, a broad-spectrum antibiotic that is specifically toxic to the insulin-producing β-cells of the

pancreas lead to diabetes of the animal model.[23,24] Lew *et al*.[25] reported that the characteristics of STZ-induced diabetes rats include hyperglycemia, polyphagia, polyuria, polydipsia, and significant weight loss. The animal model is considered diabetes if the BGL greater than 200 mg/dL. This study showed that three days after STZ induction, the rats BGL of the diabetic control group (G2) and the treatment group (G3) range from 208 to 600 mg/dL (TABLE 1).

In addition, a significant weight loss was also observed in this study after STZ induction (FIGURE 1). The significant weight loss of the STZ-induced diabetes rats occurred due to an increased protein breakdown in muscle and tissue.[26] Diabetes leads to changes in protein metabolism.[27] The elevated protein catabolism for glucogenesis that cause weight loss occurs in diabetes.[28]

In figure 3 we explained that fibrosis was observed in 8 rats of diabetic control group and the part of rats in treatment group (G3) in this study (FIGURE 3). Cardiac fibrosis is an injury condition in the cardiac muscle that is characterized by excessive deposition of type 1 collagen.[29] Cardiac fibrosis in STZ-induced diabetic rats occurs due to the differentiation of cardiac fibroblast into myofibroblast.[30] Furthermore, the myofibroblasts secrete excessive amount of ECM such as collagen.[31] It was also reported that an increased expression of TGF-β, α-SMA, and connective tissue growth factor (CTGF) that contributed in the development of cardiac fibrosis are observed in the hearts of STZ-induced diabetic rats.[30,32,33] The CTGF is a fibrogenic factor that promotes fibroblast proliferation and interstitial collagen deposition.[34]

Hyperglycemia in STZ-induced diabetic rats increased ROS production which cause imbalance between ROS and antioxidant levels lead to oxidative stress.[9,13,24,34] The levels of MDA of diabetic rats increase lead to stimulate fibroblast proliferation and other fibrotic signaling pathways in cardiac tissue.[31,34] Oxidative stress triggers the activation of the TGF-β pathway, which, in turn, activates the SMAD signaling pathway, specifically SMAD-2/3.[10] Within the nucleus, SMAD-2/3 complexes govern the transcription of target profibrotic genes responsible for encoding proteins involved in extracellular matrix production, such as collagen.[11]

This study showed that the administration of *M. oleifera* leaves extract inhibits the incidence of cardiac fibrosis in diabetic rats. A better histological feature of cardiac tissue of diabetic rats treated by *M. oleifera* leaves extract was observed compared to that untreated diabetic rats. *Moringa oleifera* leaves extract can ameliorate the histological feature of cardiac tissue of diabetic rats as almost its normal condition. Previous study reported that *M. oleifera* leaves extract has protective effect on cardiac antioxidant status and lipid peroxidation in STZ-induced diabetic rats.[35]

Moringa oleifera leaves has been reported to contain active antioxidant compounds such as vitamin C, vitamin E, and flavonoids.[36-38] Quercetin, a potent antioxidant flavonoid found in high concentration in *M. oleifera*, has therapeutic activities.[39,18] The quercetin is responsible for free radical scavenging activity. Moreover, it was also reported that the quercetin in *M. oleifera* leaves extract reduce the cardiac necrosis biomarkers levels and normalize the myocardium structure in both *in vitro* and *in vivo* stidies.[18]

Previous studies reported that *M.*

oleifera leaves extract at a dose of 1000 mg/kg BW significantly showed positive effects to the rat organs exposed to oxidative stress.[19,20,40] It was also reported that *M. oleifera* leaves extract has a renoprotective effect on the glomerulus of diabetic rats. The *M. oleifera* leaves extract restored the glomerulus damage from a score of 4 to a score of 1 to 0 or return to its normal condition. Another study reported that *M. oleifera* leaves extract has a hepatoprotective effect against oxidative stress by restoring the structure of liver tissue close to normal.[20] *Moringa oleifera* leaves extract restored the structure of pancreatic islet cells of hypercholesterolemic rats model.[40]

CONCLUSION

In conclusion, *M. oleifera* leaves extract can inhibit cardiac fibrosis in STZ-induced diabetic rats.

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Turnaround time for the provision of packed red cells (PRC) and factors affecting their achievements in the Blood Transfusion Unit of Dr. Sardjito General Hospital, Yogyakarta

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ABSTRACT

Turnaround time (TAT) is defined as the time it takes since request/sample is received at the blood bank until blood is cross-matched/reserved and available for transfusion. Turnround time prolongation affects patient care and satisfaction. This study aimed to evaluate TAT for the provision of packed red cells (PRC) at the Blood Transfusion Unit of Dr. Sardjito General Hospital, Yogyakarta, analyze factors affected in TAT prolongation, and provide solution the prolongation. It was an analytical descriptive study with a qualitative design, by calculating the time since receipt of the PRC request at the Blood Transfusion Unit or since blood collection from donors until data input of the crossmatch results in Dr. Sardjito General Hospital management information system (SIMETRIS) completed. Moreover, the delay in the provision of PRC at the Blood Transfusion Unit was also analyzed. There were 3 (1.5%) of 200 ER samples that met TAT for the provision of the PRC, which was 30 min after receipt of the request at the Blood Transfusion Unit in cito conditions. There were 20 (10%) of 200 samples from the wards that met TAT for the provision of the PRC, which was 2 h after receipt of the request at the Blood Transfusion Unit if the blood stock was available. There were 55 (27.5%) of 200 samples from the wards that met TAT for the provision of the PRC, which was 4 h after the blood was collected from the donor. TAT for the provision of the PRC at the Blood Transfusion for the available blood stock group was 179.08 (67.2 – 396.27) min, replacement blood donor group was 485.38 (126.43 – 910.68) min, and cito group was 121.29 (27.68 – 421.38) min. In conclusion, there is TAT prolongation of PRC provision at the Blood Transfusion Unit of Dr. Sardjito General Hospital.

ABSTRAK

Turnaround time (TAT) didefinisikan sebagai waktu yang dibutuhkan dari saat permintaan/sampel diterima di Bank Darah sampai darah dilakukan uji silang serasi/dicadangkan dan tersedia untuk transfusi. Pemanjangan TAT pada layanan pemeriksaan laboratorium dan Bank Darah mempengaruhi perawatan pasien serta kepuasan pasien. Penelitian ini bertujuan untuk mengetahui TAT penyediaan PRC di Unit Pelayanan Transfusi Darah (UPTD) RSUP Dr. Sardjito, Yogyakarta dan menganalisis faktor yang berperan dalam pemanjangan TAT, dan memberikan solusi permasalahan jika terjadi pemanjangan TAT. Penelitian ini merupakan penelitian deskriptif analitik dengan desain kualitatif, dengan menghitung waktu sejak penerimaan permintaan PRC di UPTD atau sejak pengambilan darah dari donor sampai pemasukan data hasil uji silang serasi di SIMETRIS selesai. Selain itu juga dianalisis jika terjadi keterlambatan penyediaan PRC di UPTD. Terdapat 3 (1,5%) sampel dari 200 sampel IGD yang memenuhi TAT penyediaan PRC yaitu 30 menit sejak SPKD diterima di UPTD pada kondisi *cito*. Didapatkan 20 (10%) sampel dari 200 sampel ruang rawat yang memenuhi TAT penyediaan PRC yaitu 2 jam sejak penerimaan SPKD di UPTD apabila tersedia stok darah. Didapatkan 55 (27,5%) sampel dari 200 sampel ruang rawat yang memenuhi TAT penyediaan PRC yaitu 4 jam sejak pengambilan darah dari donor. *Turnaround time* penyediaan PRC di UPTD RSUP Dr. Sardjito untuk kelompok stok darah tersedia adalah 179,08 (67,2 – 396,27) menit, kelompok donor darah pengganti 485,38 (126,43 – 910,68) menit, kelompok *cito* 121,29 (27,68 – 421,38) menit. Dapat disimpulkan, terdapat pemanjangan TAT penyediaan PRC di UPTD RSUP Dr. Sardjito.

Keywords:
turnaround time;
prolongation;
provision;
packed red cells;
blood transfusion unit

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INTRODUCTION

Turnaround time (TAT) is a crucial quality indicator in clinical laboratories. Timely blood supply is crucial for efficient and satisfactory laboratory services, especially during emergency operations.[1] Turnaround time is one of the 10 blood bank quality indicators. Turnaround time is defined as the time it takes from the time the request/sample is received at the blood bank until the blood is crossmatched/reserved and available for transfusion. Blood banks are required to set upper limits for routine and emergency requests separately.[2] The TAT prolongation of blood banking services affects patient care and patient satisfaction.[3] Studies reported that blood transfusion improves survival only if given immediately when needed.[4]

In terms of the definitions of TAT, a point to note is the different TAT definitions, e.g. from time of reception of a request to time at which the blood unit was handed over to the attender for transporting it to bedside, from time of request to when packed red cells (PRC) exited the blood bank, or from receipt of specimens/order in the transfusion service until units are available for issue (routine 8 h, ASAP 4 h, and STAT 2 h).[5] Because the condition of patients served in transfusion is different from routine laboratory services, the quality of service and the quality of analytics must be considered together and equally crucial. From the clinician's point of view, timely receipt of required blood units can be the most crucial performance indicator of a blood bank. In case of emergency services, a delay of just a few min in blood availability can make an overall difference. It indicates that monitoring and correcting TAT is highly recommended and beneficial for transfusion services.[2]

Regular monitoring of quality indicators including TAT in transfusion improves patient safety and customer satisfaction. The first step to reducing TAT towards the desired goal is to find out the variations of TAT for different services, products, and schedules. It is also necessary to identify the cause of the delay in TAT and take corrective action to eliminate it. The cause with the greatest effect should be addressed first, given the limited time, effort, and resources to deal with all the problems at once.[6] Root cause analysis (RCA) is a method by which researchers try to identify the underlying cause of the error or problem that caused the incident and try to correct or overcome it.[7-9]

Based on the standard operating procedure (SOP) at the Blood Transfusion Unit of Dr. Sardjito General Hospital, Yogyakarta, blood is ready to be collected within 2 h from the time of receipt of the blood components get requested if the blood stock is available. Meanwhile, if the blood still has to be collected from the donor, it is ready within 4 h. In cito conditions, the Blood Transfusion Unit must prepare blood within 30 min immediately after getting requested blood components.

From the results of interviews with internal medicine residents and nurses at the Inpatient Installation I, Dr. Sardjito General Hospital (Dahlia 1, Dahlia 2, Bugenvil 3), received information on complaints that there is often a delay in the availability of the PRC units requested, especially for cancer patients who will undergo chemotherapy protocols. To handle these complaints, the Blood Transfusion Unit must try its best to resolve the problems complained in order to maintain the trust of clinicians and the good image of the Blood Transfusion Unit itself. Complaints should be considered as indicator of agency performance appraisal, signaling some problems or failures in internal processes that require prompt resolution.[10]

MATERIALS AND METHODS

Design of study

This was a descriptive-analytic study with a qualitative design, by calculating the time since the receipt of the PRC request at the Blood Transfusion Unit (available blood stock group and cito group) or from the time of taking blood from the donor (replacement blood donor group) until the data input from the crossmatch results at SIMETRIS (Dr. Sardjito General Hospital management information system) is complete, as well as analyzing if there is a delay in the provision of PRC in Blood Transfusion Unit using Ishikawa diagrams/fish-bone analysis. This study was conducted at the Blood Transfusion Unit of Dr. Sardjito General Hospital. The time of study was carried out starting in September 2021.

Procedure

The study subjects were patients with a request for a PRC unit at the Blood Transfusion Unit of Dr. Sardjito General Hospital who met the inclusion and exclusion criteria. The inclusion criteria were all hospitalized adult patients who received PRC transfusions. The Exclusion criteria were patients who received a request for delayed PRC transfusion and patients who did not receive the first PRC from the number of PRC units requested.

Data on requests for PRC units from the Anggrek (Neuro), Bugenvil (Obsgyn), Cendana (Surgery), Dahlia (Internal Medicine), Pavilion, Intensive, Maternal wards, and Emergency Room as well as data on the provision of PRC units at the Blood Transfusion Unit of Dr. Sardjito General Hospital was taken from SIMETRIS during the period from February to May 2021. Observations and in-depth interviews were carried out with 10 blood transfusion technical officers at the Blood Transfusion Unit of Dr. Sardjito General Hospital to determine the factors that affect the TAT. The transcription and coding process was carried out from interview data.[11] The prolongation of the TAT will be evaluated by root case analysis (RCA) using Ishikawa diagrams/ fishbone analysis and solutions will be taken to solve the problems that occur.

This study used ethical clearance issued by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/ Dr. Sardjito General Hospital (Ref. No. KE/ FK/1027/EC/2021).

Data analysis

Data was presented in the mean and standard deviation (SD) if the data were considered as normal or median and the minimum-maximum value if the data were not normal. The normality of the data was carried out using the Kolmogorov-Smirnov test. Data analysis using Medcalc software version 13. Analysis and discussion of the results displayed using Ishikawa Diagram/ fishbone analysis.[12]

RESULTS

A total of 7821 PRC requests were received at the Blood Transfusion Unit of Dr. Sardjito General Hospital, Yogyakarta for the period of February to May 2021 and obtained 4132 PRC requests at the Blood Transfusion Unit of Dr. Sardjito General Hospital which met the inclusion and exclusion criteria. Based on the estimated proportion for the calculation of the sample size, a minimum sample size of 200 samples for each group was obtained according to the SOP of Dr. Sardjito General Hospital (available blood stock group, replacement blood donor group, and cito group).[13]

The characteristics of the subjects are presented in TABLE 1. The subjects in the available blood stock group and the replacement blood donor group were at

most > 60 y.o. (31% and 25%), while in the cito group, most subjects were 18-30 y.o. (29%). Female subjects were found to be dominant in the 3 groups, i.e. 63, 57, and 52% for the available blood stock group, the replacement blood donor group, and the cito group, respectively. The majority of subjects from the available blood stock group (45.5%), the replacement blood donor group (36.5%), and the cito group (44.5%) were blood type O. Two PRC units were requested by 62 (31%) subjects in the available blood stock group while 3 PRC units were requested by 63 (31.5%) subjects in the replacement blood donor group and 87 (43.5%) subjects in the cito group.

From the available blood stock group, the replacement blood donor group, and the cito group, the length of time from each ward was calculated from the time the PRC request was received at the Blood Transfusion Unit or from the time the blood was collected from the donor until the data input of the crossmatch results at SIMETRIS was completed, and analysis of the prolongation of the TAT for the provision of PRC in Blood Transfusion Unit was carried out.

TABLE 1. Characteristics of study subjects

Parameter	Available blood stock group (n=200)	Replacement blood donor group (n=200)	Cito group (n=200)
Age [years, n (%)]			
• 18-30	49 (24.5)	47 (23.5)	58 (29)
• 31-40	18 (9)	31 (15.5)	31 (15.5)
• 41-50	31 (15.5)	32 (16)	26 (13)
• 51-60	40 (20)	40 (20)	39 (19.5)
• > 60	62 (31)	50 (25)	46 (23)
Sex [n (%)]			
• Male	74 (37)	86 (43)	96 (48)
• Female	126 (63)	114 (57)	104 (52)
Blood type [n (%)]			
• A	34 (17)	39 (19.5)	43 (21.5)
• B	62 (31)	70 (35)	58 (29)
• AB	13 (6.5)	18 (9)	10 (5)
• O	91 (45.5)	73 (36.5)	89 (44.5)
Number of requests for PRC units [n (%)]			
• 1	52 (26)	21 (10.5)	6 (3)
• 2	62 (31)	58 (29)	36 (18)
• 3	46 (23)	63 (31.5)	87 (43.5)
• 4	16 (8)	11 (5.5)	24 (12)
• ≥ 5	24 (12)	47 (23.5)	47 (23.5)

Length of time for the provision of PRC

The total length of time since receiving the PRC request at the Blood Transfusion Unit or from the time of taking blood from the donor until the data input of the crossmatch results at SIMETRIS was completed for the period of February to May 2021 (TABLE 2). In the available blood stock group, the shortest time was found in the intensive ward [176.85 (77.28 – 344.42) min]. In the replacement blood donor group, the shortest period was found in the Pavilion ward [(374.23 (137.12 – 895.57)] min. In the cito group taken from the ER, the period was 121.29 (27.68 – 421.38) min. There were 3 (1.5%) samples of 200 ER samples that met the TAT for the provision of PRC according to the SOP, which was 30 min since the blood components requests were received at the Blood Transfusion Unit in cito condition.

TABLE 3 describes the minimum and maximum time for the wards from the time the PRC request was received at the Blood Transfusion Unit until the data input of the crossmatch results at SIMETRIS was completed during the period February-May 2021. There were 20 (10%) samples of 200 samples from the wards (2 samples from Bugenvil, 2 samples from Cendana, 3 samples from Dahlia, 4 samples from the Pavilion, and 9 samples from the Intensive) that met the TAT for the provision of PRC according to the SOP, which is 2 h from receiving the blood components requests at the Blood Transfusion Unit if the blood stock is available.

TABLE 4 presents the minimum and maximum time for the wards from the time of blood collection from the donor until the data input of the crossmatch results at SIMETRIS was completed for the period of February to May 2021. There were 55 samples (27.5%) of 200 samples from the wards (2 samples from Anggrek, 15 samples from Bugenvil, 11 samples from Cendana, 5 samples from Dahlia, 12 samples from Pavilion, 8 samples from Intensive, and 2 samples from Maternal) that met the TAT for the provision of PRC according to SOP, namely 4 h from the time of blood collection from the donor.

TABLE 2. The total length of time since receiving the PRC request at the Blood Transfusion Unit or since the blood collection from the donor until the data input of the crossmatch results at SIMETRIS was completed (in min) for the period of February to May 2021

Wards	Mean ± SD/median (min-max) (in min)		
	Available blood stock group (n=200)	Replacement blood donor group (n=200)	Cito group (n=200)
Anggrek (Neuro)	180.32 ± 41	525.42 ± 237.33	
Bugenvil (Obsgyn)	178.12 (67.2 – 337.17)	451.06 (126.43 – 894.78)	
Cendana (Surgery)	197.11 ± 66.67	592.25 (129.58 – 910.68)	
Dahlia (Internal Medicine)	198.62 ± 71.99	458.07 ± 234	
Pavilion	198.55 ± 73.6	374.23 (137.12 – 895.57)	
Intensive	176.85 (77.28 – 344.42)	377.22 (126.75 – 907.28)	
Maternal	228.48 ± 81.01	405.59 ± 218.65	
Emergency room			121.29 (27.68 – 421.38)

TABLE 3. The minimum and maximum time for the wards from the receipt of the PRC request at the Blood Transfusion Unit until the data input of the crossmatch results at SIMETRIS was completed (in min) during the period February-May 2021

Wards	Available blood stock group (n=200)			
	Frequency n (%)	Minimum time (min)	Maximum time (min)	TAT achievement n (%)
Anggrek (Neuro)	12 (6)	134.28	283.08	0 (0)
Bugenvil (Obsgyn)	32 (16)	67.2	337.17	2 (6.25)
Cendana (Surgery)	35 (17.5)	70.3	308.22	2 (5.71)
Dahlia (Internal medicine)	23 (6.5)	76.93	339.47	3 (13.04)
Pavilion	36 (18)	72.12	396.27	4 (11.11)
Intensive	56 (28)	77.28	344.42	9 (16.07)
Maternal	6 (3)	133.33	339.52	0 (0)

TABLE 4. The minimum and maximum time for the wards from the time of blood collection from the donor until the data input of the crossmatch results at SIMETRIS was completed (in min) for the period of February to May 2021.

Wards	Replacement blood donor group (n=200)			
	Frequency n (%)	Minimum time (min)	Maximum time (min)	TAT achievement n (%)
Anggrek (Neuro)	15 (7.5)	141.77	864.78	2 (13.33)
Bugenvil (Obsgyn)	44 (22)	126.43	894.78	15 (34.09)
Cendana (Surgery)	55 (27.5)	129.58	910.68	11 (20)
Dahlia (Internal medicine)	19 (9.5)	168.5	865.05	5 (26.32)
Pavilion	33 (16.5)	137.12	895.57	12 (36.36)
Intensive	29 (14.5)	126.75	907.28	8 (27.59)
Maternal	5 (2.5)	141.83	654.32	2 (40)

From the results of this study, it was found that most of the prolongation of the TAT in the provision of PRC between wards was from the available blood stock group, the replacement blood donor group, and the cito group. Based on the SOP at the Blood Transfusion Unit of Dr. Sardjito General Hospital, blood is ready to be collected within 2 h from the time of receipt of the blood components requests at the Blood Transfusion Unit if the blood stock is available, whereas if the blood still has to be taken from the donor, the blood is ready to be collected within 4 h from the blood collection from the donor. In cito conditions, the Blood Transfusion Unit must prepare blood within 30 min of receiving the blood components requests at the Blood Transfusion Unit.

Analysis of TAT prolongation of PRC provision

Before doing the troubleshooting, the first step that must be done is to identify

the cause of the problem. The problem that will be identified as the cause is the prolongation of the TAT for the provision of PRC in the Blood Transfusion Unit of Dr. Sardjito General Hospital. An analysis using Ishikawa Diagram was carried out to identify the cause of the problem with the approach of human resources (man), process flow (method), blood requests (material), instruments/tools (machine), samples (measurement), and blood products (milliue).

Human resources

Human resources are the main factors related to the timeliness of providing PRC at the Blood Transfusion Unit of Dr. Sardjito General Hospital, it is necessary to have an appropriate number of human resources based on workload analysis in order to not cause excessive workloads that can trigger a prolongation of the TAT. The workload analysis conducted at the Blood Transfusion Unit of Dr. Sardjito General Hospital in September 2021, obtained the number of personnel needed which was 43 blood transfusion technical personnel. The current workforce is 20 people which means there is still a shortage of 23 people.

Based on interviews conducted with 10 blood transfusion technical officers at the Blood Transfusion Unit of Dr. Sardjito General Hospital, who represented the 5 existing departments (distribution, serology, AFTAP, components and testing, and release), the problem of staff shortages became the main issue that emerged. The lack of a number of officers in each department causes the work to be carried out not optimally, such as the following interview: "If it's enough or not, it depends on what parameters we want to pursue. For example, a job must be finished, then it can be finished. But if the work must be completed in a faster time, then this is not achieved. Because if one person does multiple works at the same time he/she will need a pause or break. Thus, from the completion of the work, its effectiveness, around 70-80% of the blood from AFTAP can be processed and examined, but the time will be a bit backward than if we do each part." (Interview 02, components and testing).

In the process of taking blood from donors, the lack of staff also slows down the system, especially the night shift where there is only 1 person while the work is carried out concurrently alone. This can be seen from the following quote: "In the morning and evening shifts, there is 1 blood drawing officer and 1 hemoglobin checking officer. But in practice the admin helps check hemoglobin and there is a doctor who takes a history of the donor, so that 2 officers can focus on taking blood. On the night shift, there is only 1 officer who does everything himself: donor history, hemoglobin and blood pressure checks, blood collection." (Interview 10, AFTAP)

The incoming blood request and the requested blood collection are the responsibility of the distribution department. This department is the one that most often interacts with doctors, nurses, or families of patients who need blood. In the morning and evening shifts the number of officers is 2-3 people, while on the night shift is only 1 person on duty. The lack of human resources will increase the workload of officers and it will affect the quality of the service. A high workload will cause the timeliness of completing work to be low, as illustrated in the following quote: "In the morning and evening shifts, there are 3 people/shift: 1 operator, 1 admin, 1 technician. On Sunday mornings and evenings there are only 1 operator and 1 technician. Meanwhile, on the night shift, there is only 1 technician. The ideal number of employees is 3 people/shift both morning, afternoon, and evening shifts. The operator is in charge of answering calls from the ward and administering whatever is requested on the phone, for

example asking for blood stocks, etc. The admin is in charge of inputting the blood flask data using SIMETRIS. Technicians do checking blood type, processing of warming blood products and checking blood product labels. The new input is done when the distribution process is free for several patients at once, it can be for 10-20 patients at once. As for input, the distribution process is carried out directly every time blood is released from Blood Transfusion Unit." (Interview 06, distribution).

The serology department is the last filter before the blood is collected. The lack of staff is also a problem in the serology department because there are many stages of the process that must be carried out for a crossmatch examination in serology until the blood can be collected. "It's not enough, the problem is that 1 shift must only have 1 person. One person prepares a sample, prepares the blood that you want to crossmatch, later you have to write a label, then for example there are not enough people in front of us who will take blood sometimes, prepare our own blood, it takes too long to look for samples. Especially if you are on night shift, night shift is the most you crossmatch the sample. It's not enough, because there must be only 1 person in 1 shift. One person prepares a sample, prepares blood for crossmatch, writes a label. If there are not enough people in the distribution department, we will also collect blood, prepare blood, and look for samples. Especially if we work on the night shift, we do most of the crossmatch samples." (Interview 03, serology).

Process flow

The blood supply process at the Blood Transfusion Unit starts from the time the blood components requests are received until the blood can be collected at the distribution department. The blood components requests and samples received will then be checked for blood type and get inputted on the computer. The patient data will also be written in the blood request book and grouped by wards. The blood is ready to be collected if the blood stock has been checked and a crossmatch test is carried out in the serology department. Based on the results of the interview, it was still found that the communication system was not yet integrated between the Blood Transfusion Unit and the ward. The ward expects the blood to be processed after the blood components requests are received at the Blood Transfusion Unit, while the Blood Transfusion Unit expects confirmation from the ward for the timing of the use of blood before the blood is processed. "If there is a request from the ward for a patient who has a transfusion, we usually wait for confirmation from the ward, unless there is already a note, for example, how many hemoglobin or platelet levels are in the blood request, we usually sort it from there. If the hemoglobin level is 4 or 5, we usually process it immediately, or if the platelets are below 10,000 or low, we usually process it right away. So we are the ones who sort the blood request ourselves." (Interview 04, distribution).

In the case of a request for emergency blood, information from the distribution department will be directly forwarded to the serology department for crossmatch test before the blood is issued. This condition requires immediate treatment in order that crossmatch test is prioritized, bypassing the queue for crossmatch tests at the serology department. Based on the results of the interviews, obtained several categories of emergencies according to the distribution. "If the hemoglobin level is below 7 or the platelet level is below 50,000, we usually process it immediately. The Operating Room patient for sure. For example, someone takes blood from the Operating Room, and someone takes blood from the ward, we still prioritize those from the Operating Room. Emergency Room

too. In the ER, especially if it is confirmed immediately or if there is a note in the blood request, we will process it immediately because those from the ER, as soon as they know the laboratory results, will immediately confirm it too. That's a maximum of 30 min later the blood can come out for emergency cases like that." (Interview 04, distribution)

If the blood stock for the patient is not available at the Blood Transfusion Unit, the patient's family is recommended to find a donor or take blood at the PMI. The donor process starts with inputting donor data to SIMETRIS, checking hemoglobin and blood type, history taking, checking blood pressure, inputting data to the AFTAP computer, and taking blood. The blood that has been taken will then be processed in the component and testing department. One blood bag will be separated from the blood components, namely PRC, TC, and plasma, then the plasma will be frozen for the manufacture of FFP. After the process of separating blood components is complete, all blood components will be taken to the release department. At the same time, the EDTA sample received from the AFTAP department will be processed at the IMLTD department for screening for Hepatitis B, Hepatitis C, HIV, and syphilis. The release department will assess the feasibility and volume of the blood product as well as assess the results of the screening from the IMLTD department before determining whether the blood product can be removed. If the blood comes from a voluntary donor, then the blood product will go into the stock refrigerator, while if it is a replacement donor, the release department will look for the patient's blood components' requests first before putting the blood product into the patient's stock refrigerator. The large number of donors at the same time is a factor that prolongs the TAT of blood supply, as in the following interview: "Yes, we also work according to this flow. If it's crowded here, it must be delayed, the blood centrifugation is delayed, the result is delayed, so it takes a long time to get to the release. Depending on the intensity of the work, it's a lot or not." (Interview 05, release)

Blood requests

The blood components requests sent to the Blood Transfusion Unit are requests for blood components from a clinician that must be accompanied by the completeness of the patient's identity, doctor, and indication of transfusion. Indications of transfusion and hemoglobin information that are not filled in the blood components requests cause the Blood Transfusion Unit not to prioritize the blood request, therefore the Blood Transfusion Unit must wait for confirmation from the ward regarding when the transfusion will be performed. The wrong check on the requested blood component also causes the Blood Transfusion Unit to confirm it first to the inpatient ward before processing the blood. This can lead to a prolongation of the TAT of blood supply at the Blood Transfusion Unit. "Sometimes the blood request has a wrong tick, someone ask for PRP, it's a bit unusual. Usually, the blood request is often wrong, so later we confirm to the ward, what they want to ask is PRC or really PRP." (Interview 04, distribution)

Dr. Sardjito General Hospital is currently developing a blood request through a work order system, which is expected to speed up the process of requesting blood from the Blood Transfusion Unit. The obstacle that is still being faced is that the staff in the ward still sends blood components requests and samples to the Blood Transfusion Unit even though the blood request has been inputted in the work order, thus causing duplication of blood requests. There is still a weakness in this work order system, namely the absence of

notifications in the SIMETRIS system, causing the Blood Transfusion Unit to still have to wait for information from the ward if the ward has entered work orders and the amount of blood stock that is ready to be collected can only appear on the work order if it has been crossmatched, there are still many doctors/nurses who ask for blood stocks by telephone or come directly to the Blood Transfusion Unit.

Instruments/tools

The type of tool and maintenance of the tool will affect the process and the results carried out, which means the knowledge of the tools used and routine tool maintenance is needed, considering that the tools have the potential to be damaged. All departments at the Blood Transfusion Unit have instruments/tools, each of which has specifications and a routine maintenance schedule, either by the equipment vendor or from the IPSRS (*Instalasi Pemeliharaan Sarana Rumah Sakit*/Hospital Facility Maintenance Installation). Overall routine maintenance has been going well, whether daily, weekly, or monthly. The equipment vendors and the IPSRS are also easy to contact, and they can deal with damaged equipment immediately.

There are only a few cases of severe equipment damage that cause service interruptions, for example as described in the following quote: "Once. It happened recently. So the damage to the device is more than 24 h, resulting in blood cannot be released. All pending. We are trying too, usually we have rapid test backups, but that time is running out too. Usually if there is damage to the device for more than 24 h, we use a rapid test." (Interview 02, components and testing)

In addition to specific equipment, problematic telephone, computers, and the SIMETRIS network system can also hinder work at the Blood Transfusion Unit, resulting in delays in blood supply. Only 1 computer was placed in the distribution department, while the computer was used to input requests for entry and input blood output from the Blood Transfusion Unit, causing distribution officers to not work in parallel.

Samples

The patient's blood sample is sent to the EDTA tube together with the blood components requests, which will be used by the Blood Transfusion Unit for crossmatch and blood type examination. The input process at SIMETRIS will be carried out by the distribution department after checking the condition of the sample and checking the blood type. There are several problems with the sample that can prolong the blood supply process. "Sample without identity, identity on sample and request does not match, sample does not use EDTA tube, sample lysis, etc. If there is this problem then the sample and request are returned, not inputted first." (Interview 06, distribution)

With the transition from a manual blood request letter to a work order, it can be used to propose an installation of pneumatic tube system for transporting patient samples from the ward to the Blood Transfusion Unit. Therefore, the samples do not need to be taken to the Blood Transfusion Unit, which will shorten the service time for blood requests.

Blood products

To meet the need for blood stock, every morning the release department will look for blood products that are not used through the SIMETRIS system or telephone for confirmation to the ward. If the stock of blood and blood that cannot be used are inadequate (<10/blood type, except for AB <5/blood type),

the Blood Transfusion Unit will ask the PMI Kotagede and the PMI Sleman to drop ± 20 bags/blood type. In addition to routine stock, the Blood Transfusion Unit is also required to provide an emergency stock of at least 10 bags/blood type. The cause of the delay in providing blood products in the release department is if the family already has a donor, but after checking the request letter has run out and there is no new one, or the request letter has not yet been delivered from the ward, therefore it must be confirmed and wait again.

The release department is the department for the latest blood check at the Blood Transfusion Unit. If a problematic blood product is found, it must be re-checked which can have an impact on the time of supplying blood products, such as the following interview: "Yes, determining whether blood is worthy or not. If it's not appropriate, we won't accept it, hold on, then we'll usually trace where the error is. If it's from AFTAP, we'll fix it again, we'll keep track of it, later if we need to re-screen, we'll re-screen, we ask the IMLTD (*infeksi menular lewat transfusi darah*/ infection transmitted by blood transfusion) department to re-screen using a hose, so we don't use the sample tube." (Interview 05, release).

Reactive blood products from the results of the transfusion-transmitted infections examination will be re-screened using a blood bag tube, if the results remain reactive, the blood will be discarded and the donor status in AFTAP is blocked, for further scheduling of donor consultations at the Blood Transfusion Unit. Apart from asking for blood droppings from the PMI, another way to fulfill blood needs at the Blood Transfusion Unit is by conducting Mobile Unit activities. This method is quite effective in meeting the need for blood stock at the Blood Transfusion Unit, as explained in the following interview: "Yes, it must be Mobile Unit. With the previous Mobile Unit, it has helped to reduce the dropping. At least we must get 30 bags of blood. Yesterday we got about 26 bags of blood in the Nursing Study Program. If we have a large Mobile Unit, we can get 100 bags of blood." (Interview 05, release)

DISCUSSION

The Blood Transfusion Unit is a unit that is responsible to ensure the availability of blood for patients in need. The importance of blood availability at the Blood Transfusion Unit requires always maintaining the amount and quality of available blood to meet the need for blood transfusions. As a unit engaged in the service sector, service quality is a measure of how well the level of service provided is able to meet customer expectations.[14] The intended customer is a clinician who expects a timely blood supply. An effective problem-solving strategy involves carrying out planned activities, which can be summarized in three main steps: responding to and receiving complaints, resolving complaints, and sending feedback to customers.[10]

There was a shortage of 23 technical personnel for blood transfusion at the Blood Transfusion Unit of Dr. Sardjito General Hospital based on the workload analysis carried out. Workload analysis is a management technique that is carried out systematically to obtain information about the level of effectiveness and efficiency of an organization's work based on the volume of work. One way to calculate HR requirements is to use workload analysis with the health workload analysis method.[15]

The procurement of employees is a crucial, difficult, and complex issue, because it is used to find and place competent, compatible, and effective people. The procurement of employees requires serious attention, and is based on job analysis, job descriptions, job

specifications, and job evaluations.[16]

The lacking of officers cause the distribution of the number of officers in each department to be not ideal which is not possible to divide the officers per shift with the appropriate number and causes one department to be filled by officers from the other departments. The most workload at the Blood Transfusion Unit is on the night shift, while the duty officer is only 1 person per division which will slow down the workflow and the TAT for blood supply. With the condition of the number of human resources that is not yet ideal, the flexibility of the Blood Transfusion Unit officers is needed to be able to meet the patient's requests. Flexibility is related to the organization's ability to adapt to change.[17]

In the method factor, the thing that causes the length of the TAT for blood supply is the communication system that has not been integrated between the Blood Transfusion Unit and the ward. The ward expects the blood to be processed immediately after the blood components requests are received at the Blood Transfusion Unit, while the Blood Transfusion Unit expects confirmation of the timing of the use of blood from the ward before the blood is processed. The large number of donors at the same time is also a factor that prolongs the TAT of blood supply. Because the process of supplying blood is a flow that is interconnected between parts, delays in one part will have an effect on other parts as well. Quality patient care can only be provided if there is a combination of various disciplines working together in a unified team.[18]

The data input process in SIMETRIS which is accumulated is also a separate obstacle at the Blood Transfusion Unit. Ideally, the data input process is carried out every time the inspection is finished, but the data input process at the Blood Transfusion Unit waits for the officers to be free, especially if there is an emergency request. In conditions of emergency blood demand, the crossmatch test takes precedence, passing through the queue for the crossmatch tests at the serology department, which then the data input process will be carried out simultaneously for several patients after the officers have completed all the crossmatch examinations.

Regarding material factors, the completeness and accuracy of filling out the blood components requests, especially indications for transfusion, information on hemoglobin, and requested blood components, the Blood Transfusion Unit still has to confirm first to the inpatient ward before processing blood. The incompleteness of filling in the blood components requests is similar to the study of Ramanathan, Shaiji, and Usha which is one of the most common causes (19 cases) of prolongation of the TAT 1-5 min for emergency blood supply.[6]

The implementation of the work order system, which is expected to speed up the process of requesting blood to the Blood Transfusion Unit, has not been implemented by all wards, and there are still duplication of manual blood requests and work orders. The absence of notifications in the SIMETRIS system causes the Blood Transfusion Unit to still has to wait for information from the ward if the ward has entered a work order and the amount of blood stock that is ready to be collected can only appear on the work order if a compatible crossmatch test has been carried out, and there are still many doctors/nurses who asked for blood stock by telephone or came directly to the Blood Transfusion Unit.

All the departments at the Blood Transfusion Unit have instruments/tools, each of which already has a routine maintenance schedule on a daily, weekly, or monthly basis, either by the equipment vendor or from the IPSRS. The equipment vendors and the IPSRS

are also easy to contact, and they can deal with equipment damage immediately. The problems with telephone, computers, and the SIMETRIS network system can also hamper the work at the Blood Transfusion Unit which results in delays in supplying blood. In the study of Sharma, Arora, and Malhotra, instrument failure was the second most common cause (10 cases) of 11-15 min of TAT prolongation for emergency blood supply.[2] The prolongation of the TAT for the supply of blood could also be due to the fact that there is only 1 computer in the distribution department which is used to input requests for incoming and outgoing blood from the Blood Transfusion Unit which leads to the distribution officers cannot work in parallel.

The patient's blood sample is sent to the EDTA tube together with the blood components requests, which will be used by the Blood Transfusion Unit for crossmatch and blood type examination. If there are problematic samples, such as samples without identity, the identity on the sample and the blood request does not match, the sample does not use the EDTA tube, or the sample is lysed, then the sample and blood request will be returned to the ward first, which will extend the TAT of blood supply at the Blood Transfusion Unit. Various problems in the sample were also found in the study of Ramanathan, Shaiji, and Usha, causing a TAT prolongation of ≥ 20 min for blood supply in emergency conditions.[6]

The availability of safe blood in hospitals is one of the Hospital Minimum Service Standards, which means that every hospital must have a 24-h safe blood stock at the Hospital Blood Bank or Hospital Blood Transfusion Unit.[19] If the stock of blood and blood that cannot be used is insufficient, then the Blood Transfusion Unit will ask the PMI Kotagede and the PMI Sleman to drop ± 20 bags/group of blood. If the patient's family already has a donor, but after checking the letter of request for blood has run out and there is no new one, or the letter of request for blood has not been delivered from the ward, then the Blood Transfusion Unit must first confirm to the ward before being able to provide blood for the patient. In the case of problematic blood product conditions, such as barcode discrepancy and reactive transfusion-transmitted infections screening results, the Blood Transfusion Unit must also re-check which can have an impact on the time of supplying blood products.

The Comprehensive Blood Transfusion Unit management is needed to address the various causes of the problem of prolonging the blood supply TAT. According to Rusdiana, the management function has task characteristics, namely planning, organizing, mobilizing, and controlling, which can be interpreted as a systematic process carried out by superiors in an agency to achieve goals. During the implementation of management functions, employees will raise their personal expectations of agency policies. Whether or not employees' expectations with the agency are in accordance with the agency's expectations will be reflected through employee perceptions of the management function that it will shape the expected performance.[20] It is in line with the study conducted by Kalkavan and Katrinli which states that the optimal performance of employees is influenced by employee perceptions of their responsibilities.[21]

Based on the results of the analysis carried out in the study, it was found that several problems were the cause of the delay in providing PRC at the Blood Transfusion Unit Dr. Sardjito General Hospital. All problems cannot be solved at the same time because each problem has a different and complex cause. Problem identification and problem-solving priorities are necessary steps for

policy formulation in order that real and relevant problems can be identified and the most urgent and crucial problems will be addressed first.[22] In addition to proposing the addition of the number of officers to the HR of Dr.Sardjito Hospital, the Blood Transfusion Unit accelerated the implementation of automation and carried out regular Mobile Unit activities to meet the amount of blood stock.

The changes that will be made by the Blood Transfusion Unit is aiming to improve the service quality of the Blood Transfusion Unit as one of the work units in Dr. Sardjito General Hospital. The service quality is the result of a comparison between customer expectations (expected quality) and customer experience when experiencing a service (experienced quality). If the perceived service is as expected, then the service quality is perceived as good and satisfactory. Whether or not the quality of the service is good depends on the ability of service providers to meet the customer expectations.[23]

CONCLUSION

Turnaround time for the provision of PRC at the Blood Transfusion Unit of Dr. Sardjito General Hospital for the available blood stock group is 179.08 (67.2 – 396.27) min, the replacement blood donor group is 485.38 (126.43 – 910.68) min, the cito group is 121.29 (27.68 – 421.38) min. It is found that there is TAT prolongation of PRC provision in the Blood Transfusion Unit of Dr. Sardjito General Hospital. The factors that play roles in the prolongation of the TAT for the provision of PRC are: man (lack of technical personnel for blood transfusion, 1 person on duty per division on night shifts), method (unintegrated communication system between the Blood Transfusion Unit and wards, large number of donors at the same time), materials (incomplete and inaccurate filling of the blood components requests, the implementation of the work order system has not been carried out by all wards, no notification in the SIMETRIS system if the inpatient ward has input work orders, the number of patient blood stocks can only appear on the work order if a compatible crossmatch test has been carried out), machine (telephone, computers, and the SIMETRIS network system is problematic, only 1 computer is placed in the distribution department), measurement (samples are problematic), milleu (inadequate blood stock, the blood request letter has run out or the request letter for blood has not been delivered from the ward, the condition of the blood product is problematic).

Furthermore, it can be suggested to propose additional blood transfusion technical officers that it can reduce the shortage of officers at the Blood Transfusion Unit, automatisation of crossmatch examinations which the results can be automatically inputted into SIMETRIS, improvement of the SIMETRIS system which leads work order notifications appear on SIMETRIS and show the amount of blood stock available for patients, implementation of work orders simultaneously for all wards to avoid duplication of blood requests, the completeness and accuracy of filling out the blood requests and sending EDTA samples from the ward, installing a pneumatic tube system for transporting EDTA samples from the ward to the Blood Transfusion Unit, add 1 unit of computer in the distribution department in order to speed up the process of inputting requests for incoming and outgoing blood from the Blood Transfusion Unit, and implementation of routinely scheduled Mobile Unit activities to ensure the availability of blood stock at the Blood Transfusion Unit.

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Comorbidities of COVID-19 patients associated with mortality at the Baubau Regional Public Hospital, South East Sulawesi

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that cause coronavirus disease 2019 (COVID-19) has become a global pandemic. Until November 30th, 2020, World Health Organization (WHO) confirmed 61,869,330 positive cases with 1,448,896 deaths (CFR 2.3%). Some comorbidities are associated with the COVID-19 mortality. This study aimed to investigate risk factors of the COVID-19 mortality at the Baubau Regional Public Hospital, Sout East Sulawesi. It was a cross-sectional study with a retrospective analysis involving 81 COVID-19 patients. Purposive sampling was applied in this study. Chi-square analysis was conducted to calculate odd ratio (OR). The result showed that in the period from January to September 2021, 30 COVID-19 patients died consisting of 20 male and 10 female. Most of the patients died were >45 yo and only 4 patients died were <45 yo. Among the patients died, 11 patients had hypertension, 12 patients had type 2 diabetes mellitus (DM), 4 patients had pulmonary TB and 3 patients had dyspepsia. Further analysis showed that hypertension (OR=6.803; 95%CI: 1.925-24.038; p=0.002) and dyspepsia (OR=0.222; 95%CI: 0.059-0.838; p=0.016) were significantly associated with the COVID-19 mortality, whereas type 2 DM (OR=1.123; 95%CI: 0.445-2.832; p=0.495) and pulmonary TB (OR=0.559; 95%CI: 0.059-0.838; p=0.270) were not. In conclusion, hypertension is risk factor, whereas dyspepsia is protective factor of COVID-19 mortality.

ABSTRAK

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) yang menyebabkan penyakit *coronavirus disease 2019* (COVID-19) telah menjadi pandemi global. Hingga 30 November 2020, Organisasi Kesehatan Dunia (WHO) mengonfirmasi 61.869.330 kasus positif dengan 1.448.896 kematian (CFR 2,3%). Beberapa penyakit penyerta dikaitkan dengan kematian akibat COVID-19. Penelitian ini bertujuan untuk mengkaji faktor risiko kematian akibat COVID-19 di RSUD Baubau Sulawesi Tenggara. Penelitian ini merupakan penelitian potong lintang dengan analisis retrospektif yang melibatkan 81 pasien COVID-19. Sampling purposive dilakukan dalam penelitian ini. Analisis chi-kuadrat dilakukan untuk menghitung *odd ratio* (OR). Pada periode Januari hingga September 2021, pasien COVID-19 meninggal sebanyak 30 orang yang terdiri dari 20 laki-laki dan 10 perempuan. Pasien meninggal terbanyak berusia >45 tahun dan hanya 4 pasien meninggal berusia <45 tahun. Pasien meninggal tersebut terdiri dari 11 pasien menderita hipertensi, 12 pasien menderita diabetes melitus (DM) tipe 2, 4 pasien menderita TBC paru, dan 3 pasien menderita dispepsia. Analisis lebih lanjut menunjukkan bahwa hipertensi (OR=6,803; 95%CI: 1,925-24,038; p=0,002) dan dispepsia (OR=0,222; 95%CI: 0,059-0,838; p=0,016) berhubungan secara signifikan dengan kematian akibat COVID-19, sedangkan DM tipe 2 (OR=1,123; 95%CI: 0,445-2,832; p=0,495) dan TB paru (OR=0,559; 95%CI: 0,059-0,838; p=0,270) tidak. Kesimpulannya, hipertensi merupakan faktor risiko, sedangkan dispepsia merupakan faktor protektif terhadap kematian akibat COVID-19.

Keywords:
COVID-19;
dyspepsia;
hypertension;
mortality;
risk factor

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) emerged in China in December 2019 and has since spread over the world. In severe patients with comorbidities, COVID-19 can cause death.[1,2] The World Health Organization (WHO) declared the COVID-19 outbreak a public health emergency of worldwide concern on January 30th, 2020, and a pandemic on March 11th, 2020.[3] It was reported around 20-51% of the patients had comorbidities such as hypertension, diabetes, cardiovascular, and other cerebrovascular.[4-6]

The first pathological findings of COVID-19 was obtained from a lung tissue biopsy sample of a patient died due to SARS-CoV-2 infection. The most common pathology of COVID-19 is pneumonia which may be accompanied by acute respiratory distress syndrome (ADRS) and can be fatal. COVID pneumonia is a potentially life-threatening complication that is most common in older patients, and patients with obesity, hypertension and diabetes.[6,7] These pathological findings can help physicians to identify a cause of death and formulate a therapeutic strategy to reduce mortality.[7]

In Indonesia, the first case of COVID-19 was reported on March 2nd, 2021 and then the cases are increasing and spreading rapidly throughout Indonesia. The Ministry of Health reported 70,736 confirmed cases of COVID-19 on July 9th, 2020, with 3,417 mortalities (CFR 4.8%). According to the National Task Force for COVID-19 Handling, the most positive confirmed cases (30.8%) were in the age range of 31-45 years, whereas the least occurred in the age range of 0-5 years (2.5%). Patients over 60 years of age would have the greatest fatality rate (43.3 percent). Males account for 56.7% of all death cases, while females account for 43.5%. Hypertension (11.8%), diabetes mellitus (10.6%), heart disease (6.9%), and renal disease (3%) are among the other causes of mortality.[8]

The Provincial Health Office of South East Sulawesi reported there were 6,502 confirmed cases on November 30th, 2020, with 106 deaths (CFR 1.63%). The Baubau Regional Public Hospital is one of the COVID-19 referral hospital in the province. This study aimed to investigate the risk factors of the COVID-19 mortality in the Baubau Regional Public Hospital. The risk factors included age, gender, and comorbidities were analyzed in this study.

SUBJECTS AND METHODS

Population and study design

This retrospective observation research used medical record from March to August 2021. Patients confirmed positive for COVID-19 who came to the Baubau Regional Public Hospital were gathered. The comorbidities of COVID-19 include hypertension, pulmonary TB, type 2 DM and dyspepsia were then listed.

Protocol of study

The diagnosis of COVID-19, type 2 DM, hypertension, and dyspepsia was taken from the medical records. The diagnosis of COVID-19 was carried out based on clinical examination using Swab Antigen and PCR in the hospital laboratory, then continued by reviewing the results. Patients with COVID-19 positive having comorbidities such as hypertension which had been diagnosed by a cardiologist, type 2 DM and dyspepsia by an internist, and co-morbidities with pulmonary TB by a pulmonologist were recruited as study sample. The protocol of the study was approved the Medical Research Ethic Board, Faculty of Medicine and Health Science, University of Bengkulu, Indonesia (ref. no. 2041N30.14.9 tLT 12022).

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows Version 20.0 (IBM Corp. Armonk, NY, USA). The univariate analysis was used to describe the characteristics of subjects. The bivariate analysis using the chi-square was used to calculate the odds ratio (OR) representing the association between potential risk factors of the COVID-19 mortality. If the OR value >1 indicates increased occurrences of a mortality and if OR value <1 indicates decreased occurrences of a mortality. A p value <0.05 was considered significant.

RESULTS

The characteristics of patients with COVID-19 at Baubau Regional Public Hospital are presented in TABLE 1. Male patients were higher than female patients. Patients aged >45 yr were higher than ≤45 yr. Most patients with COVID-19 did not have comorbidities.

Hypertension (p=0.002) and dyspepsia (p=0.016) were significantly associated with the COVID-19 mortality, whereas type 2 DM (p=0.495) and pulmonary TB (p=0.270) were not significantly associated with the COVID-19 mortality at the Babau Regional Public Hospital (TABLE 2). The COVID-19 patients with hypertension experienced approximately 6.8 times the risk of mortality (OR=6.803; 95%CI: 1.925-24.038), whereas the COVID-19 patients with dyspepsia experienced approximately 0.2 times the risk of mortality (OR=0.222; 95%CI: 0.059-0.838).

TABLE 1. The characteristic of patients with COVID-19 at Baubau Regional Public Hospital

Variable	n (%)
Gender	
• Male	51 (63.0)
• Female	30 (37.0)
Age (yr)	
• ≤ 45	19 (23.5)
• > 45	62 (76.5)
Hypertension	
• Yes	15 (18.5)
• No	66 (81.5)
Type-2 DM	
• Yes	31 (38.3)
• No	50 (61.7)
Pulmonary TB	
• Yes	15 (18.5)
• No	66 (81.5)
Dyspepsia	
• Yes	20 (24.7)
• No	61 (75.3)
Total	81 (100)

TABLE 2. Analysis of risk factors for COVID-19 mortality included hypertension, type 2 DM, pulmonary TB, and dyspepsia

Comorbid	Died	Survive	Total	OR (95% CI)	p
	n (%)	n (%)	n (%)		
Hypertension					
• Yes	11 (73.3)	4 (26.7)	15 (100)	6.803 (1.925–24.038)	0.002
• No	19 (28.8)	47 (71.2)	66 (100)		
Total	30 (37.0)	51 (63.0)	81 (100)		
Type 2 DM					
• Yes	12 (38.7)	19 (61.3)	31 (100)	1.123 (0.445–2.832)	0.495
• No	18 (36.0)	32 (64.0)	50 (100)		
Total	30 (37.0)	51 (63.0)	81 (100)		
Pulmonary TB					
• Yes	4 (26.7)	11 (73.3)	15 (100)	0.559 (0.161–1.946)	0.270
• No	26 (39.4)	40 (60.0)	66 (100)		
Total	30 (37.0)	51 (63.0)	81 (100)		
Dyspepsia					
• Yes	3 (15.0)	17 (85.0)	20 (100)	0.222 (0.059 – 0.838)	0.016
• No	27 (44.3)	34 (55.7)	61 (100)		
Total	30 (37.0)	51 (63.0)	81 (100)		

DISCUSSION

Among the risk factors investigated in this study, only hypertension was associated with the COVID-19 mortality (OR=6.803; 95%CI: 1.925-24.038; p=0.002). The COVID-19 patients with hypertension experienced approximately 6.8 times the risk of mortality compared to without hypertension or normotension. Previous studies reported that COVID-19 patients with hypertension are at higher risk of mortality and developing severe disease.[9-12] It was reported that hypertension is more prevalent in COVID-19 patients who lead to the primary endpoint and severe disease.[9] Moreover, COVID-19 patients with cardiovascular disease and hypertension had a higher mortality rate as compared to patients that without comorbid.[10] A meta-analysis included 24 observational studies reported that hypertension is associated with a significantly increased risk of COVID-19 mortality in hospital.[11] However, another meta-analysis reported that hypertension is not an independent risk factor for in-hospital mortality when adjusted for other comorbidities in hospitalized COVID-19 patients.[12]

Several hypotheses have been postulated to explain of the association between hypertension and COVID-19 mortality. Hypertension is more frequently experienced in elderly patients and it is often associated with other comorbidities such as cardiovascular disease, diabetes, and respiratory disorders. These comorbidities can contribute to COVID-19 severity and mortality.[9,12,13] Hypertension plays an important role in the regulation of renin-angiotensin-aldosterone system (RAAS), inflammation, immune responses, and the gastrointestinal tract which

partly explain in COVID-19 severity and mortality.[14]

The RAAS is key regulator of blood volume and vascular resistance in the body. Angiotensin-converting-enzyme 2 (ACE2) is an important component of the RAAS in the heart, kidney, and lung. In the other hand, ACE2 receptor is the main target of the SARS-CoV-2 virus to enter human cells.[15] Hypertension is associated with an increase in ACE2 receptor expression, potentially providing more entry points for the virus leading to promote viral interaction with host cells and exacerbating COVID-19.[15-17]

Hypertension activates the innate and adaptive immune systems, leading to cytokine release and enhanced inflammation. In hypertensive patients, the amounts of circulating monocytes, macrophages, CD8+ T cells, and CD4+ T cells are increased in the inflammatory environment.[18,19] High blood pressure promotes an acute cardiac inflammatory response and induces immune cell infiltration and activation in the myocardium that promote cardiac or other organ damage in patients with severe COVID-19.[14] SARS-CoV-2 infection also activates both innate and adaptive immune responses, triggers release of proinflammatory factors, and results in hyperinflammation or cytokine storms which can lead to ADRS and multiple organ failure (MOF).[14,20] The combination of pre-existing inflammation from hypertension and the hyperinflammation as well as immune responses to the SARS-CoV-2 infection can contribute to COVID-19 severity.

In contrast with hypertension, this study showed that dyspepsia was not the risk factor of COVID-19 mortality. The COVID-19 patients with dyspepsia only experienced approximately 0.2 times the risk of mortality (OR=0.222; 95%CI: 0.059-0.838) compared to without dyspepsia. With OR value < 1, it is indicated that dyspepsia is the protective factor of COVID-19 mortality. The protective factor of dyspepsia has been reported previously. Inversely, it was reported that COVID-19 pandemic negatively affected functional dyspepsia or irritable bowel syndrome. The COVID-19 is important independent factor associated with deterioration in gastrointestinal symptoms.[21] However, during COVID-19 quarantine an improvement of the majority of upper gastrointestinal symptoms was reported.[22] Further study is needed obtain conclusive evidence linking dyspepsia directly to COVID-19 mortality.

CONCLUSION

In conclusion, hypertension is risk factor of COVID-19 mortality at the Baubau Regional Public Hospital, South East Sulawesi. In contrast, dyspepsia is protective factor of COVID-19 mortality in this study. Further study should be conducted to obtain conclusive evidence linking dyspepsia directly to COVID-19 mortality.

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A rare case of *Salmonella* sp septic arthritis in a patient with systematic lupus erythematosus (SLE)

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ABSTRACT

Septic arthritis is considered as a medical emergency which can lead to significant morbidity and cause substantial mortality, especially if the diagnosis is delayed. Prolonged use of immunosuppressive and cytotoxic medications as therapy for systemic lupus erythematosus (SLE) causing patient susceptible to secondary infection. However, septic arthritis due to *Salmonella sp.* is very rare, makes this is an important extraintestinal manifestation especially in immunosuppressed patients. We presented a case of 25 y.o. female diagnosed with SLE 3 m.o. earlier presented with fever and arthritis on her left genu for 1 wk duration. Genu ultrasonography showed synovitis genu sinistra with fluid volume of 1-2 cc on recessus lateral genu sinistra. The patient was further analysis on her synovial fluid was conducted, the gram stained smear of the fluid showed >25 leucocytes, low power field, and *Salmonella sp.* was isolated from her synovial fluid analysis. The patient was given intravenous ciprofloxacin and discharged home well. Septic arthritis should always be considered in any patients on long-term immunosuppression state who present with acutely swollen joints. It considered as an important medical emergency with high mortality and morbidity. Hence, prompt recognition, joint aspiration with administration of systemic antibiotics and appropriate surgical intervention plays a pivotal role to minimize morbidity and mortality.

ABTRAK

Artritis septik merupakan suatu kegawatdaruratan medis yang dapat menyebabkan morbiditas dan mortalitas yang signifikan terutama bila diagnosis terlambat ditegakkan. Penggunaan terapi imunosupresif dan sitotoksik dalam jangka waktu panjang sebagai terapi lupus eritematosus sistemik (LES) dapat menyebabkan pasien lebih rentan mengalami infeksi sekunder. Namun, artritis septik yang disebabkan oleh *Salmonella* sp. sangatlah langka. Hal ini menjadi manifestasi ekstraintestinal yang penting terutama pada pasien dengan imunokompromis. Kami melaporkan seorang pasien perempuan berusia 25 tahun yang terdiagnosis dengan LES 3 bulan sebelumnya. Pasien mengalami demam dan artritis pada genu kiri selama 1 minggu. Ultrasonografi genu menunjukkan adanya sinovitis pada genu sinistra dengan volume cairan 1-2 mL pada reccesus lateral genu sinistra. Selanjutnya, dilakukan analisis cairan sinovial dimana pewarnaan Gram menunjukkan adanya >25 leukosit pada perbesaran rendah dan ditemukan *Salmonella* sp. pada cairan sinovial. Pasien mendapatkan terapi siprofloksasin intravena. Pasien akhirnya dapat keluar dari rumah sakit setelah dinyatakan sembuh. Adanya artritis septik harus selalu dipertimbangkan pada pasien dengan terapi imunosupresif jangka panjang yang mengalami pembengkakan sendi akut. Artritis septik dianggap sebagai kegawatdaruratan medis penting dengan mortalitas dan morbiditas yang tinggi. Oleh karena itu, diagnosis dini, aspirasi sendi, pemberian antibiotik sistemik, dan intervensi pembedahan yang tepat memegang peranan penting untuk menurunkan morbiditas dan mortalitas pada kasus artritis septik.

Keywords:
septic arthritis;
systemic lupus erythematosus;
Salmonella;
case report;
immunosuppression

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INTRODUCTION

Septic arthritis is a rapid and progressive infection caused by invasion of bacteria, virus or fungus into the synovial joint.[1] It is known to be the most threatening of the multiple causes of acute joint pain.[2] The incidence of septic arthritis is between 2 to 6 cases per 100,000 people but varies based on the presence of risk factors.[3] It is considered as an important medical emergency which can be associated with significant morbidity and can cause substantial mortality.[2]

Systemic lupus erythematosus (SLE) is regarded as the most common underlying disease due to avascular necrosis.[4] The prevalence of SLE varies between 4 and 27%.[5] Prolonged use of immunosuppressive and cytotoxic medications as therapy for SLE causing patient susceptible to secondary infection.

In all age and risk groups, the most frequent causative organisms identified are *Staphylococcus aureus* followed by other gram positive bacteria, including *Streptococci.*[6] *Salmonella pyogenic* infection on joints only occurs in less than 1% of cases, that makes this is a rare yet important extraintestinal manifestation.[7]

Septic arthritis caused by *Salmonella* is frequently unidentified in early phase of the disease due to its unspecific symptoms and signs. A delay in diagnosis or inadequate treatment is not infrequent, which can lead to irreversible joint distruction and increasing mortality.[2] This case report indicates the importance of prompt recognition and timely diagnosis through synovial fluid aspiration of relevant joints, the choice of suitable antibiotics, and appropriate intervention to restrain permanent disability particularly in immunosuppressed patient.

CASE

A 25 y.o. woman was admitted via the emergency room presented with fever 1 d prior to admission and worsening pain on her left knee for a wk duration. The pain was non radiating, severe in intensity with a scale 8 out of 10 and aggravated by movement at the knee. The patient did not have any history of trauma, prior surgery, and there were no accompanying symptoms or pain and swelling in any other joint of the body. The patient was diagnosed with SLE 3 m.o. before admission, characterized by malar rash, oral ulcer, proteinuria, and arthritis. She was maintained with methylprednisolone 16 mg q.d., mychophenolate mofetil 500 mg b.i.d. and had not reported any symptoms during the previous months.

Upon admission, she was compos mentis, with a blood pressure 110/60 mmHg, cardiac rate 110 times/min, respiratory rate 22 times/min, and temperature of 37.8 ^{o}C. The physical examination on her left genu showed a swollen, erythematous knee, which was warmth, oedema and tender on palpation with decreased range of motion of the knee flexion and extension due to pain. The remainder of the physical examination was within normal limits. Laboratory examination showed hemoglobin levels 12.4 g/dL, leukocytes 15.26× 10^{6}/L. Renal function tests, electrolytes and liver enzymes were normal.

A consideration of secondary septic arhtritis was made as she was in immunosuppressed state. Before

the results of the synovial fluid culture and sensitivity test, the patient was started empirically with intravenous ceftriaxone 2 g q.d., daily medications were continued. Afterwards, she underwent further examination, joint aspiration was performed. Her synovial fluid analysis revealed yellow color, cloudy, with 80,000 erithrocyte/mm^3, 58,750 leucocytes/mm^3 and negative for monosodium urate monohydrate. The Gram stained smear of the fluid showed >25 leucocytes, low power field, there was no Gram negative dipolococcus bacteria aspirated from her synovial fluid, and the culture results found *Salmonella sp.* growth which was sensitive to ceftazidime and ciprofoloxacin.

Ultrasonography was performed and it showed synovitis genu sinistra with fluid volume of 1-2 cc on reccesus lateral genu sinistra **(**FIGURE 1**)**.

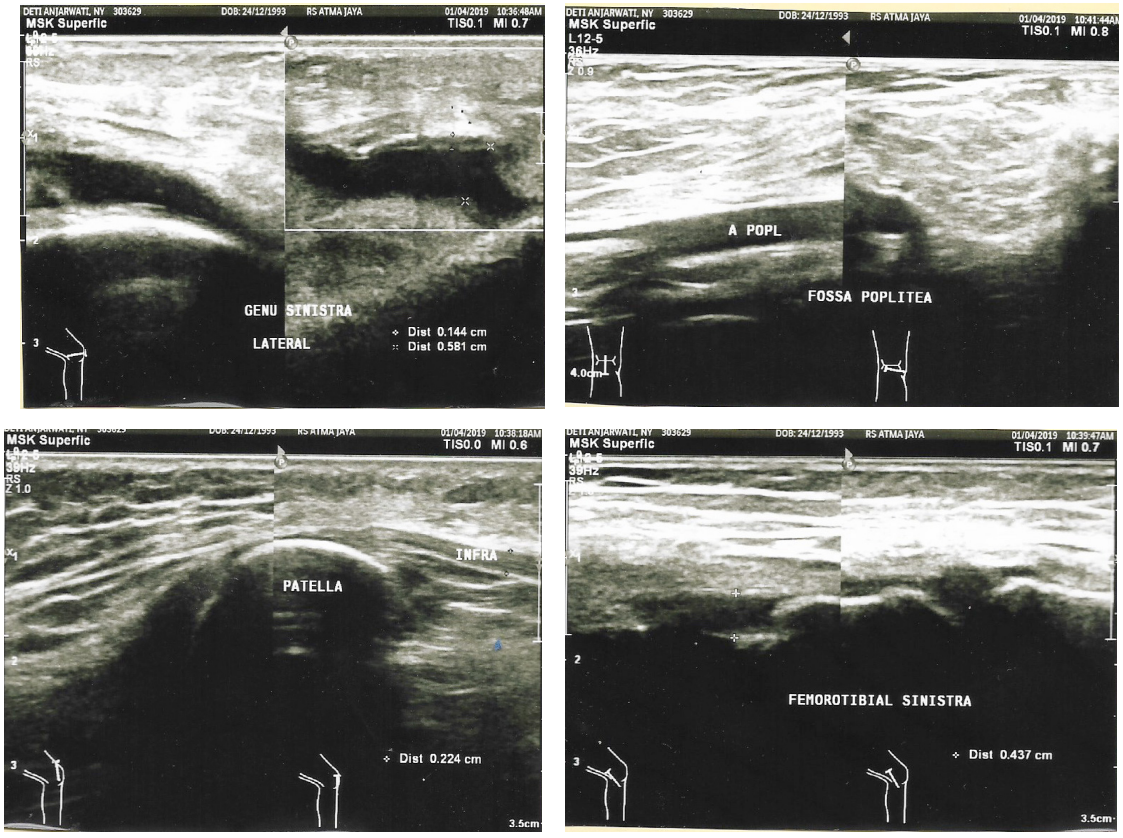

FIGURE 1. Ultrasound of the genu sinistra which showed synovitis with fluid volume of 1-2 cc on reccesus lateral genu sinistra

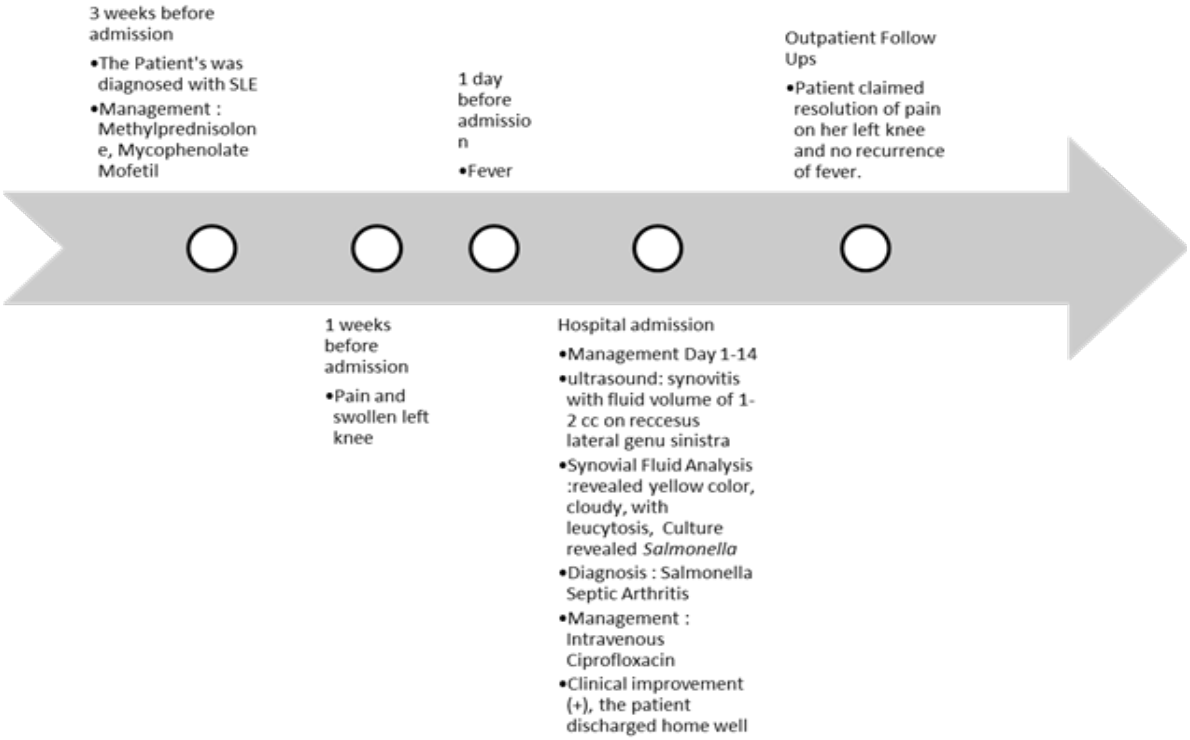

FIGURE 2. Timeline of case

Based on the findings obtained, all results established the diagnosis of *Salmonella* related septic arthritis. On account of the culture results, her antibiotic changed to intravenous ceftazidime and ciprofloxacin for 2 wk before switching to oral cefixime and ciprofloxacin to be continued for 2 wk. Duration of treatment in studies ranged from 4 to 6 wk, split into 1-2 wk of intravenous antibiotics, depending on patient's response, and a further 3–4 wk of oral antibiotics.[7] She was monitored closely; surveillance aspirations of knees had negative microbiology cultures. Clinically, gradual improvement of patient's arthritis was noted and was then discharged home well. Her outpatient follow-up examinations for up to 6 m.o. were uneventflul with no complaints of join pain and no recurrence of febrile episodes. A case timeline of the patient's history, clinical manifestation, management, and follow up are summarized in FIGURE 2.

DISCUSSION

Infection can have fatal consequences and accounts for 25% mortality in patients with SLE.[8] In patients treated with immunosuppressant medication, pulmonary, opportunistic and septic arthritis are the most common infections.[9] Septic arthritis is known to be the most threatening of the multiple causes of acute joint pain, and can be associated with significant morbidity, including permanent joint dysfunction.[4,10] It has long been viewed as an orthopedic

emergency as it can lead to significant morbidity and even mortality.[10] In adults of all age groups with septic arthritis, *S. aureus* is the most common causative organism, followed by group A *Streptococcus, Haemophilus influenzae* type b, *Streptococcus pneumoniae*, and *Brucella melitensis*[1,2,10] *Salmonella sp.* (including typhoidal and non-typhoidal strains) arthritis is atypical and accounts for only 1% of all cases.[1,7,8,11,12] The most common *Salmonella sp.* serotypes isolated from these SLE patients were type B and D, and *S.enteritidis* was the most common pathogen causing septic arthritis in younger SLE patients.[4,14]

Salmonella consists of a large heterogeneous group of gram negative bacilli, this intracellular organism may act as a carrier and causes widespread infection in the immunosuppressed state.[8,11,14] It can be easily eradicated in healthy individuals, but it causes widespread infections in immunosuppressed patients, infection can spread through hematogen and present as focal lesions in any organ with or without suppuration and associated with gastrointestinal infection, but immunosuppression leads to an increased risk of bacteremia, endovascular infection, soft-tissue abscesses, and bone and joint involvement.[8,11,15]

Several authors have addressed predisposing factors that associated with *Salmonella*-related septic arthritis, these factors included: connective tissue diseases (15.5%), HIV (18.6-20%), malignancy (23.6-36.4%), diabetes mellitus (29.5%), immunosuppressive conditions, chronic kidney diseases, atherosclerosis and hypertension (27.9-69.1%), age > 65 yr, gastrectomy, chronic lung diseases, rheumatoid arthritis, amyloidosis, immunosuppressive therapy (steroids, cytotoxic chemotherapy), leukemia and lymphoma, and thalassemia.[10,12,16,20] SLE is regarded as the most common underlying disease in patients with Salmonella bacteremia.[7,12] Self-limited gastroenteritis and bacteremia, with or without extra-intestinal focal infections (EFIs), are common clinical presentations of nontyphoidal salmonellosis[5,18] *Salmonella typhi* septic arthritis occurs less frequently, tends to affect adolescents and young adults and has predilection for the left sacroiliac joint.[4,7] The etiopathogenesis of AVN in SLE can can be caused by multiple factors, such as high-dose or long-duration steroid therapy, immunosupressant therapy, positivity for antiphospholipid antibody, lupus nephritis, neuropyschiatric lupus, and cushingoid status.[5] High susceptibility to *Salmonella* infection in SLE patients may be due to hypocomplementemia, a phagocytosis defect, defective turnout necrosis factor production, increased haemolysis, a cellular immune defect, immunosuppressant drugs, incomplete antibiotic use and glomerulonephritis.[8]

Salmonella septic arthritis developed within 1-53 m.o. after SLE diagnosis. The reactivation of latent infection and new infection may both be possible routes. A common predisposing articular factor for Salmonella septic arthritis is avascular necrosis (AVN) and osteonecrosis (ON).[8,11,15] The complications of AVN, cellular immune defect by steroid use and alcoholic liver disease may induce a disturbance of the osseous arterioles and even microvascular tamponade, which then becomes a local factor for *Salmonella* infection and provided a favorable environment for persistent bone infection following an episode of bacteraemia.[9,11]

Smith *et al*.[19] reported that enzymatic destruction begins by the 8 h after the inoculation. By the 48th h, 40% of the glycosaminoglycan is lost, and collagen breakdown occurs in a period of few days in septic arthritis.[19] Nierenberg *et al*.[2] previously reported a case of young woman diagnosed with SLE, end stage renal disease on hemodialysis (ESRD), and chronic joint pain that progressed into rapid septic shock within hours. This proves that prompt and timely diagnosis of the septic joint is very important, but the time course is usually considered to be within the day, and the notion of the "golden hour" for septic shock is not often considered.[2] Septic arthritis should always be treated as a potential septic emergency and diagnosis and institution of definitive therapy needs to be started in a time frame corresponding to sepsis, especially in the immunocompromised patient.[9]

The gold standard of treatment is joint debridement and antibiotic therapy according to the culture results. *Salmonella* is mostly sensitive to fluoroquinolones and third generation cephalosporins.[1-10,12,19,20] Duration of treatment in studies ranged from 4 to 6 wk, split into 1-2 wk of intravenous antibiotics, depending on patient's response, and a further 3–4 wk of oral antibiotics. Early initiation of effective antibiotics has been reported to result in good clinical response.[7-9]

In our patient, the presence of immunosuppressed state act as predispose factor for *Salmonella* septic arthritis infection due to avascular necrosis or osteonecrosis. We believe she might have had an earlier subclinical gastrointestinal infection, and it presents as a result of hematogenous spread. Appropriate antibiotic therapy and joint aspiration should be administered immediately. In most septic arthritis, *Salmonella* is not suspected as common etiology and the diagnosis is established following its isolation. Therefore, empiric therapy of septic arthritis should be targeted against *S. aureus* and *Streptococci*, in conjunction with *Salmonella* should taken into consideration particularly in immunocompromised patient.[10] Definitive therapy for septic arthritis is based on the identification and antibiotic susceptibility of the bacteria isolated in the synovial fluid culture.[20]

CONCLUSION

Septic arthritis should always be considered in any patients with immunosuppression state who present with acutely swollen joints. *Salmonella* is the most common pathogen particularly in younger SLE patients. In our patient, the SLE and use of glucocorticoids would have created an immunocompromised state which become major predisposition for *Salmonella* infection. Prompt recognition, joint aspiration and microbiology should always be obtained with prolonged administration of systemic antibiotics and appropriate surgical intervention play a pivotal role in successful treatment.

ACKNWLEDGEMENTS

No conflict of interest is declare.

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Coronavirus disease 2019 (COVID-19) related stroke incidence: a case series

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ABSTRACT

Coronavirus disease 2019 (COVID-19) can cause systemic and respiratory symptoms. Acute respiratory distress syndrome (ARDS), anemia, acute heart injury, secondary infection, and stroke are the complications of COVID-19. Age, oxidative stress, endothelial dysfunction, inflammatory status, vascular risk factors, and hypoxemia are risk factors for stroke associated with COVID-19. In this case report, two cases of COVID-19 complicated by stroke and other thromboembolic diseases were discussed. Case 1: a 46-year-old man presented with right extremities weakness, dysarthria, cough, colds, chest pain radiating to left upper extremity. He was diagnosed with moderate COVID-19, with complication of embolic stroke and myocardial infarction. After administration of IV furosemid and recombinant tissue plasminogen activator (rTPA), his condition improved, and he was discharged from our facility. Case 2: a 54-year-old woman presented with a decreased level of consciousness, skin discoloration, tenderness on her left calf, cough, fever, and shortness of breath. She was diagnosed with moderate COVID-19 with a complication of thrombotic stoke and deep vein thrombosis (DVT). She was treated with rTPA, IV citicoline, and fondaparinux for 5 days. In conclusion, COVID-19 carries a risk of thromboembolic complication. COVID-19 patients have a higher risk of bleeding, therefore, medications, particularly anticoagulant, should be administered with more caution.

ABSTRAK

Coronavirus disease 2019 (COVID-19) dapat menyebabkan gejala sistemik dan gejala pernafasan. Komplikasi yang bisa dialami pasien dengan COVID-19 adalah sindrom distres napas akut, anemia, cedera jantung akut, infeksi sekunder, dan stroke. Faktor risiko terjadinya stroke pada COVID-19 meliputi usia, stres oksidatif, disfungsi endotel, status inflamasi, faktor risiko vaskular, dan hipoksemia. Dalam laporan kasus ini, kami membahas dua kasus COVID-19 yang mengalami komplikasi stroke dan penyakit tromboembolik lainnya. Kasus 1: laki-laki 46 tahun datang dengan kelemahan anggota gerak tubuh bagian kanan, disartria, demam, dan nyeri dada yang menjalar ke tangan kiri. Pasien didiagnosis COVID-19 derajat sedang dan mengalami komplikasi stroke emboli serta infark miokardium. Setelah pemberian furosemid dan *recombinant tissue plasminogen activator* (rTPA) IV, kondisi klinis pasien membaik dan kemudian pulang dari rumah sakit. Kasus 2: wanita 54 tahun datang dengan penurunan kesadaran, perubahan warna kulit dan bengkak pada betis kana, batuk, demam, dan sesak. Pasien didiagnosis dengan COVID-19 derajat sedang, dengan komplikasi stroke trombotik dan trombosis vena dalam. Pasien dirawat dengan rTPA, citicolin IV, dan fondaparinux selama 5 hari. Kesimpulan, COVID-19 memiliki risiko komplikasi tromboembolik. Pasien COVID-19 juga rentan mengalami perdarahan, sehingga diperlukan kehati-hatian dalam pemberian terapi, terutama antikoagulan.

Keywords:
COVID-19;
stroke;
SARS-CoV-2;
risk factors;
thromboembolic

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an emerging disease mainly manifested as respiratory symptoms. However, it also causes systemic symptoms, with approximately 36.4% of patients experiencing neurological manifestations, including headache, impaired consciousness, and paresthesia. These symptoms are more common in patients with severe cases, compared to those with mild or moderate COVID-19.[1] Several studies hypothesize that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, binds to angiotensin converting enzyme 2 (ACE2) receptors on brain tissue.[2]

Coronavirus disease 2019 also causes coagulopathy, inflammatory cascade, hypoxia, and vascular endothelial dysfunction, which increase the risk of thrombus formation. These processes are thought to be responsible for thrombotic complications of COVID-19, including deep vein thrombosis (DVT), pulmonary embolism (PE), myocardial injury, and ischemic stroke. Compared to other thrombotic complication of COVID-19, stroke is considered rare.[1,3,4] In this case series, two cases of COVID-19 infection with complication of stroke, one of which also had myocardial injury, and DVT on another case were presented. In-depth discussion of coagulopathy and thrombotic complication of COVID-19 were also provided.

CASES

Case 1

A 46-yo male, with known history of hypertension, dyslipidemia, and ischemic stroke in 2018, presented to the emergency department with sudden worsening of left upper and lower extremity weakness and worsening dysarthria for 4 h before admission. He also had a cough, cold, chest pain radiating to left upper extremity, and shortness of breath for 3 d. A screening test with rapid COVID-19 antigen was positive, and polymerase chain reaction (PCR) test confirmed the COVID-19 diagnosis.

In the emergency department, he was somnolent with a Glasgow Coma Scale (GCS) E4V5M4, elevated blood pressure (171/92 mmHg), tachycardia (heart rate 122 bpm), tachypnea (respiratory rate 26 bpm), elevated temperature (37.9°C), and hypoxia (SpO2 93% on room air). Coarse crackles were found throughout both lung fields. Extremities drifted to the left on physical examination, indicating left sided weakness, with normal tone in all extremities. Physiological reflexes increased, and left Babinski reflex was observed, indicating upper motor neuron (UMN) lesion.

Blood work revealed electrolyte imbalance (calcium ion 0.95 mmol/L), coagulopathy (D-dimer 3044.59 ug/L), dyslipidemia (total cholesterol 256 mg/dL), and increased cardiac biomarker (HS troponin I 60 ng/L). A chest X-ray revealed bronchopneumonia, and a head CT scan showed hypodensity on the right pons, thalamus, and posterior limb of internal capsule, supporting the diagnosis of multiple lacunar infarction (FIGURE 1). An electrocardiography (ECG) study showed ST elevation on lead II, III, and aVF, indicating inferior ST elevation acute coronary syndrome (STE-ACS).

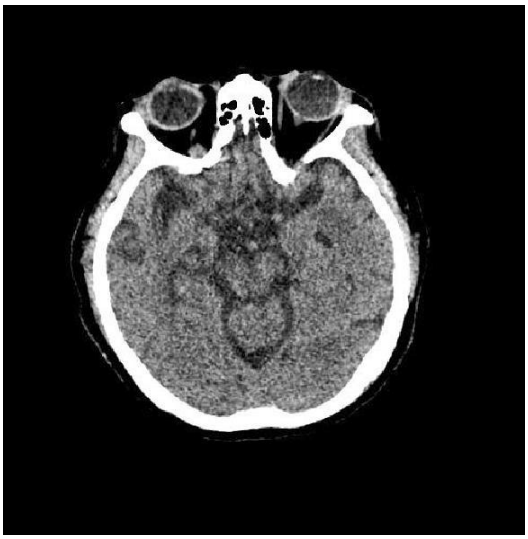

FIGURE 1. Multiple lacunar infarction on pons, thalamus, and posterior limb of internal capsule

A diagnosis of moderate COVID-19 with complication of embolic stroke and myocardial infarction was made, and IV furosemide 2 0mg q.d. was administered for pulmonary edema. Recombinant tissue plasminogen activator (rTPA), a thrombolytic agent, is administered in doses of 40 mg, 10% as an initial IV bolus over one min and the reminder infused over 60 min. Citicoline is given intravenously in doses of 250 mg twice daily as a neuroprotectant to stabilize membrane permeability. Hypertension was managed with oral carvedilol 6.25 mg b.i.d., and oral ramipril 5 mg q.d., targeting 160/90mmHg on 6 h. IV ciprofloxacin 400 mg b.i.d., IV vitamin D 2000 IU q.d., IV paracetamol 500 mg q.i.d., oral favipiravir 600 mg b.i.d, and oral ambroxol 30 mg t.i.d. were administered following the Indonesian recommendation for COVID-19 infection. Evaluation PCR test on day 8 and 9 of treatment showed negative COVID-19 results, and she was discharged from isolation room. She then moved to non-infection ward for further evaluation. After 11 d of treatment, she showed improvement of left extremities weakness, and she was discharged from our facility.

Case 2

A 54-yo female was brought to the emergency department with decreased level of consciousness, skin discoloration, and tenderness on her left calf. For four days before admission, she had a cough, fever, and shortness of breath.

On physical examination, a decreased level of consciousness with GCS E4V4M5, elevated blood pressure (143/82 mmHg), tachycardia (heart rate 108 bpm), tachypnea (respiratory rate 22 bpm), elevated temperature (38.1°C), hypoxia (SpO2 94% on room air), and coarse sounds in both lung fields were observed. On neurological examination, her extremities drifted to the left, indicating lateralization to the left. An increased muscle tone, increased physiological reflexes, and a left Babinski reflex, indicating an UMN lesion were also observed. On palpation, the left Gastrocnemius muscle was swollen, warm, darkened, and the total Well's score was 3, suggesting DVT (TABLE 1).

TABLE 1. Wells' criteria for DVT

Clinical feature	Score
Active cancer, treatment or palliation within 6 mo	0
Bedridden recently >3 d or major surgery within 12 wk	1
Calf swelling >3 cm compared to the other leg, measured 10 cm below tibial tuberosity	1
Collateral (non-varicose) superficial veins present	0
Entire leg swollen	0
Localized tenderness along the deep venous system	1
Pitting edema, confined to symptomatic leg	1
Paralysis, paresis, or recent plaster immobilization of the lower extremity	0
Previously documented DVT	0
Alternative diagnosis to DVT as likely or more likely	0
Total Score	4

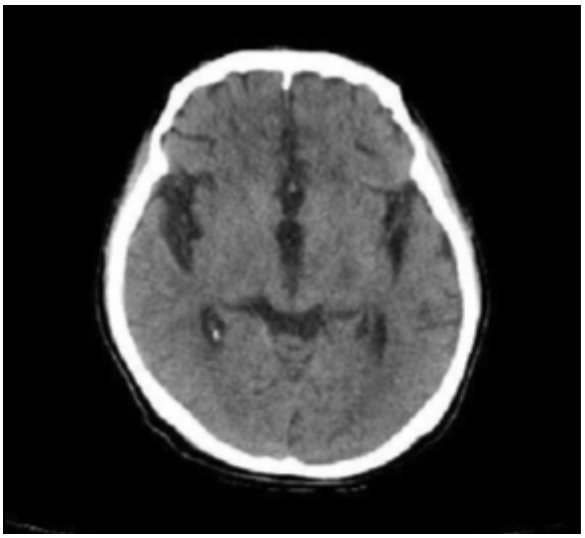

FIGURE 2. Hypodensity lesion in right hippocampus and temporal horn of internal capsule

Laboratory results showed a hypercoagulable state (D-dimer 4,904.43 ng/mL), leukocytosis (leukocytes 16,810/μL, absolute lymphocyte count 1,300/μL, lymphocytes 8.1%, and neutrophils 84.2%). A chest X-ray revealed infiltrate in both lung fields, increased bronchovesicular markings, indicating bronchopneumonia. A head CT scan revealed a hypodensity lesion in the right hippocampus and temporal horn of the internal capsule, indicating ischemic stroke (FIGURE 2). On ultrasonography study, we found total occlusion of popliteal vein (FIGURE 3).

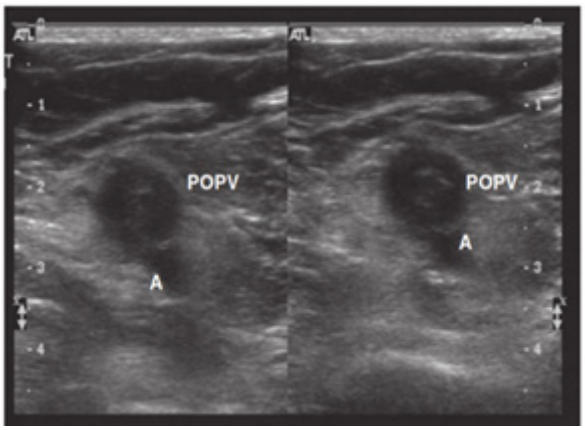

FIGURE 3. Total occlusion of popliteal vein

A diagnosis of moderate COVID-19 with complications of thrombotic stroke and DVT was made. She received 40mg of rTPA, administered as 10% initial IV bolus over 1 min and the reminder infused over 60 min, IV citicoline 250 mg b.i.d., and fondaparinux 2.5 million IU q.d. for 5 d. For the management of moderate COVID-19, she received favipiravir 600 mg b.i.d., IV paracetamol 1 g b.i.d, IV dexamethasone 5 g q.d., and IV ciprofloxacin 400 mg b.i.d. Her level of consciousness improved to E4V3M5 after 5 d of treatment and to E4V5M6 1 d. After her COVID-19 PCR test converted to negative on 8th day of treatment. She was transferred to a non-infectious ward. Once she was fully conscious, we observed mild paresis of the left VII and XII nerves, upper limb strength of (555/222), and lower limb strength of (555/222). She was discharged after showing improvement in upper and lower limb strength to (555/333) and (555/333), respectively; also, pain and inflammation on the left calf subsided on 11th day of treatment.

DISCUSSION

As of February 2022, COVID-19 cases reached 392 million, with more than 5.7 million deaths globally. According to epidemiological studies, patients with acute COVID-19 infection are at risk for thromboembolic complications.[5] DVT is the most common type (around 46%) of thromboembolic complication of COVID-19, followed with pulmonary embolism (24%), myocardial injury (20%), and stroke (1.6%).[6]

In this study, 2 cases of stroke as a complication of COVID-19 were represented. Myocardial infarction occurred in one patient, and DVT in another case were also found. In these cases, thromboembolic complications were observed in moderate COVID-19, rather than severe infection. Neurological complications in COVID-19 infection may result from direct central nervous system infections or abnormalities due to the parainfective process. Several mechanisms have been proposed to explain comorbidities between COVID-19 infection and stroke, including endotheliopathy, hypoxia, and inflammatory cascade.[2,7-9]

A retrospective study from Wuhan, China showed that the incidence of stroke in COVID-19 patients was around 5%.[10] Stroke (especially ischemic stroke) was more common in patients with severe infection than in patients with mild infection (5.7% vs. 0.8%).[11] Stroke and severe COVID-19 share common risk factors, such as hypertension, diabetes, coronary heart disease, and chronic kidney failure.[12] However, COVID-19 patients with thromboembolic complication do not always have classical

risk factors like atrial fibrillation, family history of venous thromboembolism (VTE), or antiphospholipid antibody syndrome. Some COVID-19 patient still develop thromboembolism, even after receiving anticoagulants prophylaxis.[13]

Similar to vascular endothelial dysfunction with sepsis-induced coagulopathy, endotheliopathy appears to contribute to the pathophysiology of microcirculatory changes in SARS-CoV-2 infection. SARS-CoV-2 binds to ACE2 receptors on endothelial cells, and its replication causes inflammatory cell infiltration, endothelial cell apoptosis, and microvascular prothrombotic effects. These alteration by SARS-CoV-2 infection will lead to endothelial cell dysfunction.[14]

Endothelial cell dysfunction due to infection, including SARS-CoV-2 infection, leads to an accumulation of thrombin production and decreased fibrinolysis, creating a hypercoagulable state, and increasing the risk of thromboembolism complication.[2] Proliferation of SARS-CoV-2 in lung tissue causes diffuse alveolar and interstitial edema and exudate, forming a transparent membrane. This process leads to disruption of alveolar gas exchange, which in turn causes hypoxia in the central nervous system and increase in anaerobic metabolism in the mitochondria of neural cells. Acid accumulation as a byproduct of anaerobic metabolism could further lead to cerebral vasodilation, neural cell edema, interstitial edema, obstruction of cerebral blood flow, cellular damage, and apoptosis due to ischemia and congestion.[3] Additionally, hypoxia that occurs in severe COVID-19 infection can trigger thrombosis, not only by increasing blood viscosity but also by hypoxia-inducible transcription factor-dependent pathways.[4,8]

Coronavirus disease 2019 infection is also associated with proinflammatory cytokines that can induce mononuclear and endothelial cell activation, which in turn causes activation of coagulation and thrombus formation. The circulation of free thrombin, which cannot not be controlled by natural anticoagulants, can lead to platelet activation and cause thrombosis. Severe inflammatory status is characterized by an increase in CRP and D-dimer, which indicates a disorder of the coagulation cascade that plays a role in the hypercoagulability status of COVID-19 patients.[9] Hypoxia also triggers the infiltration of inflammatory cells and the release of cytokines, which in turn contribute to tissue ischemia. Inflammation plays an important role in the development and prognosis of cerebrovascular disease. Patients with severe infections typically exhibit higher plasma cytokine levels, such as IL-2, IL-7, IL-10, GSCF, IP10, MCP1, and MIP1A. The inflammatory process also promotes atherosclerosis and affects plaque stability.[3]

Coronavirus disease 2019 infection can also cause hemorrhagic stroke, particularly in patients older than 60 yo. Older individuals often present with more severe systemic symptoms, such as thrombocytopenia, and in addition to increased levels of D-dimer, would increase the risk hemorrhagic stroke. Furthermore, cytokine storms and predominant sympathetic activation in COVID-19 also increase the risk of aneurysms and vascular rupture, which may turn into hemorrhagic stroke.[3,15]

The concept of protected code stroke (PCS) has been introduced to provide effective care for stroke patients during the COVID-19 pandemic.[16] The PCS recommends that paramedics screen for COVID-19 infection in all patients with stroke-like symptoms before taking them to hospital. All healthcare professional exposed to patients are required to use personal protective equipment (PPE), including full-sleeve gown, surgical mask, head covering, face shield, and gloves. External transfer should be minimized, and patients who required

to be transferred should be screened for infection.[16]

The COVID-19 pandemic also posed some difficulties in examining patients. In many centers, although exhibiting signs and symptoms of stroke, patients could not receive proper treatment due to limitations in radiological examination. Fortunately, in our facility, a separate CT-scan for COVID-19 is available; thus, no patients are neglected from treatment.

Early anticoagulant administration for COVID-19 infection is recommended by Chinese authorities to improve clinical outcome, but no specific inclusion or exclusion criteria have been established. Low-molecular-weight heparin (LMWH) is widely used to prevent disseminated intravascular coagulation (DIC) and venous thromboembolism (VTE). LMWH also has anti-inflammatory effects, which may benefit COVID-19 patients. Complication of LMWH administration, such as bleeding, are rare and usually mild when they occur. Expert consensus suggests that LMWH prophylaxis should be administered upon admission and continued for 7-14 days after discharge. For severe COVID-19 patients with coagulopathy, unfractionated heparin or LMWH is recommended to reduce coagulation substrate depletion. In critically ill patients, LMWH or un-fraction heparin (UFH) is preferable to oral anticoagulants due to their shorter half-life [4,17] In a series of five cases of large vessel stroke in COVID-19 patients, antiplatelets were initially administered, but later switched to anticoagulants. Anticoagulant prophylaxis with LMWH is recommended for stroke cases with COVID-19 infection. Early anticoagulation with LMWH may be useful in reducing the risk of thromboembolism in COVID-19-associated stroke patients, while considering the risk of intracranial hemorrhage or the transformation of infarct stroke into hemorrhagic.[18]

In the Indonesian guideline of COVID-19, prophylaxis with LMWH or UFH is recommended for moderate and severe COVID-19.[19] None of our cases received any anticoagulant prophylaxis, as they arrived at our facility after developing thromboembolic complication.

The SARS-CoV-2 specifically binds to the ACE2 receptor, which may increase the risk of intracranial hemorrhage in patients with hypertension. Critically ill COVID-19 patients also tend to have severe thrombocytopenia, another risk factor for cerebral hemorrhage. Therfore, antihypertension therapy is essential for COVID-19 patient with hypertension comorbidity. ACE inhibitors or angiotensin receptor blockers (ARBs) are not recommended as antihypertensive drugs. Other classes of antihypertensive medications, such as calcium channel blockers (CCBs), diuretics, and others, should be considered for treating hypertension in COVID-19 infection.[20]

CONCLUSION

In this case series, two cases of COVID-19 with stroke as thromboembolic complication of the infection was reported. One patient also had myocardial injury, while another had DVT. Anticoagulants play an important role in preventing those events, but since stroke in COVID-19 patients carries an increased risk of bleeding, these medications should be administered with more caution.

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Complete recovery of severe coronavirus disease 2019 (COVID-19) infection in an obese patient

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SARS-COV-2;
metabolic syndrome;
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immune function

ABSTRACT

There is strong evidence indicating that excess adiposity in obesity impacts immune function and host defence. However, almost no known mechanism of how the immune and host defence are affected by the low-grade inflammatory response of the obese has been established. The significance of altered immune response in obesity was presumed to be an independent risk factor for increased morbidity and mortality following the influenza pandemic back in 2009. Similarly, obesity is linked with a higher risk of severity and a worse clinical outcome of severe acute respiratory coronavirus 2 (SARS-COV-2) infection.This case reports a complete recovery of a severe coronavirus disease 2019 (COVID-19) infection despite having morbid obesity aggravated by metabolic syndrome.

ABSTRAK

Penelitian menunjukan jaringan lemak berlebih pada obesitas menyebabkan perubahan fungsi sistem imun dan pertahanan tubuh. Akan tetapi, masih belum diketahui pasti bagaimana inflamasi derajat rendah pada obesitas mempengaruhi sistem imun serta pertahanan tubuh. Perubahan respon imun pada pasien obes dinilai merupakan faktor risiko independen morbiditas dan mortalitas pandemi influenza tahun 2009. Demikian juga, obesitas dinilai meningkatkan risiko infeksi *severe acute respiratory coronavirus 2* (SARS-COV-2) yang lebih berat serta luaran klinis yang lebih buruk. Telah dilaporkan pemulihan sempurna pasien *coronavirus disease* 2019 (COVID-19) derajat berat dengan komorbid obesitas berat dan sindrom metabolik.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory coronavirus 2 (SARS-CoV-2) has emerged as a global pandemic since December 2019. According to WHO, by May 2021, there have been 160 million confirmed cases worldwide, 1.72 million of which are specifically found in Indonesia.[1,2]

Obesity is a common metabolic disorder which has a rising pattern worldwide.[3] Nearly 650 million people are affected by obesity, of which roughly 1.2 million are found in Indonesia. Obesity is characterised by a state of low-grade, chronic inflammation in addition to disturbed levels of circulating nutrients and metabolic hormones.[4]

COVID-19 appeared to have a strong connection between those with obesity and the risk of hospitalization, needing treatment in intensive care units (ICUs) and even death.[2,5] Despite all the poor

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prognosis either by many proposed mechanism and research evidence, we present a case of a morbidly obese patient with severe COVID-19 infection with a complete recovery.

CASE

A 32-y.o. man presented with shortness of breath and headache accompanied by non-productive cough was brought to the emergency department. The headache was first experienced six days earlier and were accompanied by breathlessness which worsen with mild physical activity, altered sense of smell and the feeling of generalized weakness. Physical examination revealed laboured breathing with respiration rate thirty times per minute, oxygen saturation of ninety five percent using non rebreathing mask (NRM) with fifteen litres of oxygen, blood pressure of 148/68, heart rate of 110 times/min, temperature of 36.8 ^{o}C and body mass index (BMI) of 41.5. Laboratory result revealed elevated C reactive protein (CRP), elevated lactate dehydrogenase (LDH) and lymphocytopenia thus resulting in AIFFEL COVID-19 scoring system of high probability. A positive polymerase chain reaction (PCR) swab was taken revealing cycle threshold (CT) value of 24.50 (Reference range: positive if < 36.0). Chest x-ray revealed severe bilateral pneumonia typical of COVID 19 appearance with normal heart size (FIGURE 1.A). Hence, severe COVID-19 infection, stage 1 hypertension, obesity and metabolic syndrome diagnosis was established given its physical examination, laboratory and radiological workup.

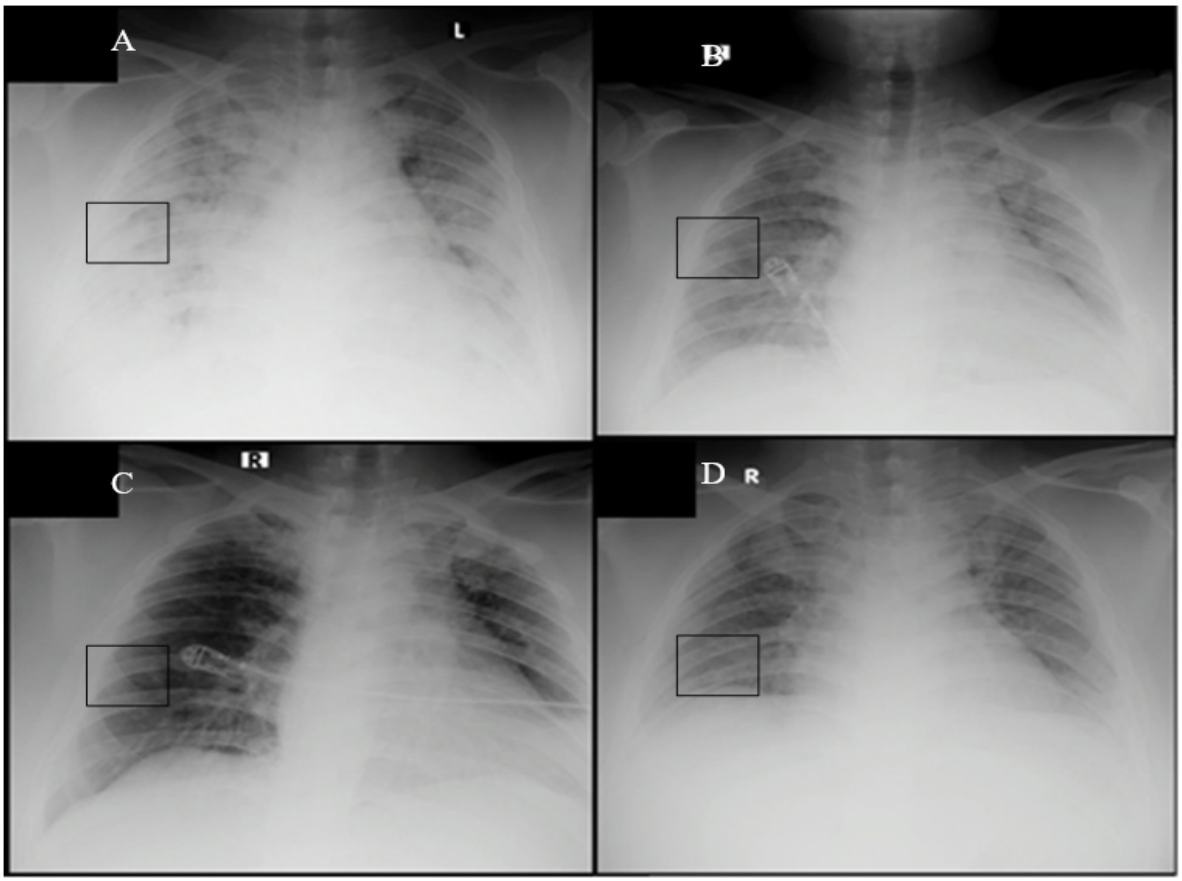

FIGURE 1. Serial Chest X-ray. (A) Chest X-ray taken on 1st day of admission (B) Chest X-ray taken on 5th day of admission (C) Chest X-ray taken on 8th day of admission (D) Chest X-ray taken on 13th day of admission.

The patient was admitted to the intensive isolation ward and was given high flow nasal cannula (HFNC) of 100/60 for oxygen therapy instead of using NRM. Meropenem 1 g/8 h and levofloxacin 750 mg/d was given empirically. Loading dose (200 mg/d) of remdesivir was given intravenously and was followed by maintenance dose (100 mg/d), enoxaparin was given twice daily subcutaneously with dose adjustment of 120 mg every 12 h, 6 mg of dexamethasone once daily, 5 mg of amlodipine taken once daily for blood pressure control and other supportive adjuvant agents such as intravenous N-acetylcysteine 5 g/d, vitamin C 1 g/d, vitamin D 400 mg/12 h, zinc which were given in order to treat the patient. On the fourth day of hospitalization, oxygen therapy was down titrated using HFNC of 70/60 based on blood gas analysis parameters. Chest x-ray evaluation revealed severe bilateral pneumonia typical of COVID-19 appearance with signs of radiological improvement compared to the previous result on the fifth day of hospitalization (FIGURE 1.B), On the sixth day of hospitalization, staphylococcus aureus was identified in the sputum culture revealing meropenem and levofloxacin resistance, hence vancomycin 1 g/12 h was initiated based on the sensitivity test. Blood culture did not reveal any grown organism. On the seventh day of hospitalization Chest x-ray evaluation revealed signs of radiological improvement compared to its previous one (FIGURE 1.C). Oxygen therapy was down titrated the following day with HFNC 60/30 and nasal cannula of 4 L/min respectively, vancomycin injection was switched into trimethoprim-sulfamethoxazole 960 mg every 12 h orally. On the eleventh day, the patient was transferred to the non – intensive isolation ward. The patient was treated for three more days and no event was recorded. Radiographic thorax image was evaluated revealing signs of radiological deterioration compared to the previous radiological chest image (FIGURE 1.D) The patient was discharged and was planned to be assessed five days later in the pulmonary clinic. His discharge medications include cotrimoxazole 960 mg/12 h, amlodipine 10 mg/d, zinc 20 mg/d, vitamin C 250 mg/d, and vitamin D 400 mg/12 h. Laboured breathing, fatigue and headache were denied during follow up and no complaints were reported.

DISCUSSION

Individuals suffering from obesity were more at risk for COVID-19 positive, for hospitalization, for ICU admission, and for mortality.[2] There is a clear relationship between obesity and basal inflammatory status characterized by higher circulating IL-6 and CRP levels. Adipose tissue in obesity is proinflammatory, with increased expression of cytokines and particularly adipokines.[6] Obesity is characterized by adipose tissue expansion and affects the inflammatory response. Adipocytes secret pro-inflammatory cytokines, such as IL-1, IL-6 and IL-10, which results in elevated circulating levels of cytokines and chemokines in the plasma of obese patients.[8] The function of various immune cells is also altered in obese patients, which significantly affects the immune system. Laboratory findings suggest that the number of lymphocytes including CD4+, T cells, CD8+ T cells, B cells, and natural killer (NK) cells are dramatically decreased in COVID-19 patients. Unfortunately, obesity impairs both T and B cell responses, therefore retards the adaptive immune response to infection. The weakened immune system in obese patients may result in higher viral load, rapid viral replication and spreading.[7]

Despite suffering from obesity, this patient eventually had a full recovery its symptoms of severe COVID-19 infection. We proposed several reasons

which might be use as a rationale to determine the prognosis of the patient. We may consider CT value as one of the prognostic variables along with other biomarkers. Lower CT value might be associated with increased ICU admission, higher mortality and increased length of ICU stay.[9] Haematological parameters specifically lymphopenia is associated with disease severity, in which the case we present showed normal laboratory parameters particularly leucocyte count and normal distribution of lymphocyte. Patients who have died from COVID-19 have had significantly lower lymphocyte counts than survivors.[10]

Pulmonary function parameter measured by blood gas analysis (BGA) namely the P/F ratio shows improvement within the first week in ICU survivors as opposed to non-survivors.[11] This finding is in line with the case we report which showed an early improvement of P/F ratio on the third day of care. Long COVID-19 was characterized by symptoms of fatigue, headache, breathlessness and anosmia and was more likely with increasing age, BMI and female gender.[12-14] There are five symptoms experienced during the first week that were most predictive of long COVID-19, the symptoms are fatigue, headache, breathlessness, hoarse voice and myalgia.[15] This case report presents a man with all the symptoms mentioned excluding hoarseness and still completely recuperated despite obesity and had a chest x-ray deterioration one day before discharge.

CONCLUSION

Obesity is linked with poorer outcome compared to lean body mass. Despite all the theory and mechanism explaining its worse outcome, the patient in this reported case was completely recovered from severe COVID-19 infection. A prospective study with a large specific sample might be required to establish and prognosticate the relationship between obesity and COVID-19 infection.

STATEMENT OF ETHICS

Written informed consent for publication of their details was obtained from the patient himself. Institutional approval was not required to publish this case report.

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CONFLICT OF INTERESTS

The author declares no conflict of interests

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Gut dysbiosis and the role of probiotics in chronic kidney disease

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ABSTRACT

Chronic inflammatory condition in chronic kidney disease (CKD) patients is associated with increased risk of cardiovascular morbidity and mortality. Gut dysbiosis is assumed as one of leading factors to the chronic inflammatory condition. The relationship between the kidney and the gastrointestinal, knowns as the gut-kidney axis, has a role in production and accumulation of uremic toxins derived from gut microbial fermentation of protein, and translocation of endotoxins and microbial from gut lumen into bloodstream due to alterations of intestinal epithelial barrier in CKD patients. Probiotics supplementation is one of the optional theraphy to restore the gut dysbiosis in CKD patients. Recent studies found that probiotics supplementation in CKD patients decreased uremic toxins and pro-inflammatory cytokines production, and delayed CKD progression. The improvement of this chronic inflammatory condition is expected to decrease cardiovascular disease risk in CKD patients. This review aims to describe the importance of gut-kidney axis in CKD patients, particularly in gut dysbiosis, and the role of probiotics in progression of CKD.

ABSTRAK

Kondisi inflamasi kronik pada penderita penyakit ginjal kronik (PGK) berhubungan dengan peningkatan risiko morbiditas dan mortalitas kardiovaskular. Salah satu sumber penyebab timbulnya inflamasi kronik ini adalah perubahan bakteri usus. Adanya hubungan antara ginjal dan saluran cerna pada penderita PGK ini disebut aksis usus-ginjal, yang berperan dalam hal produksi dan akumulasi toksin uremik hasil fermentasi protein oleh mikroflora usus, serta translokasi endotoksin dan mikroba dari lumen usus ke aliran darah akibat gangguan barier epitel usus. Salah satu alternatif memperbaiki kondisi disbiosis usus adalah dengan pemberian probiotik. Beberapa studi yang ada saat ini, menunjukkan bahwa pemberian probiotik pada penderita PGK dapat menurunkan produksi toksin uremik dan sitokin inflamasi, serta memperlambat progresi PGK. Dengan perbaikan kondisi inflamasi ini diharapkan akan menurunkan risiko penyakit kardiovaskular pada penderita PGK. Tujuan dari tinjauan ini adalah untuk memaparkan pentingnya aksis usus-ginjal pada penderita PGK, khususnya pada kondisi disbiosis mikrobiota usus, dan peran probiotik dalam menghambat perkembangan PGK.

Keywords:
gut dysbiosis;
chronic kidney disease;
probiotics;
gut-kidney axis;
chronic inflammation

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INTRODUCTION

Chronic kidney disease (CKD) is a global health problem with the prevalency around 11-13% in adults, that majority are on stage 3.[1] All stages of CKD are related to an increased risk of cardiovascular morbidity, early mortality, and decreased quality of life.[1,2] Cardiovascular disease (CVD) is the most common cause of mortality in CKD patients, and the risk of mortality is not only associated with the common risk factors such as hypertension, diabetes, and dyslipidemia. Moreover, it also associated with non-conventional risk factors, such as chronic inflammation.[2-4]

Chronic inflammation in CKD or end-stage kidney disease (ESKD), whether it is related to dialysis or non-dialysis factors, is caused by the increased production of pro-inflammatory cytokines and decreased renal clearance, the interaction between the blood and dialyzer, unsterilized dialysis fluid, infection (primarily bacterial), inadequate intravenous iron supplementation, and other comorbidities such as heart failure.[3]

Several studies reported a strong relationship between gut and kidney in CKD. Although the clinical infection has not been observed, the typical inflammatory condition in CKD could be induced by the gut translocation of molecules and pro-inflammatory cytokines or gut microbiota translocation from gut into the vascular. Thus, the innate immunity is triggered by the bacteria structures, such as the lipopolysaccharides from the gram-negative bacteria cell wall and creates inflammation.[5] Meanwhile, recent studies showed that uremia can affect the structure and functions of the gut barrier, and influence the inflammation in CKD.[3,5-8]

This review aims to show the importance of the gut-kidney axis in CKD patients, especially gut dysbiosis, and the role of probiotics in inhibiting the progression of CKD.

MATERIAL AND METHODS

Literature searches were performed on PubMed and Google Scholar without any restrictions. Free words and Medical Subject Headings (MeSH) were used to construct the search terms related to gut dysbiosis, probiotics, and chronic kidney disease. Eligible articles for further review were identified by screening of titles and abstracts, followed by a full-text review. In the case of multiple publications, the most recent and complete reports were included. Authors finalized the literature search on January 31, 2021.

RESULTS

A total of 75 articles from PubMed and 103 articles from Google Scholar were obtained at the start of the search. Sixty-four articles were removed due to duplication. We screened abstracts and conclusions of each literature. Sixty-eight literatures were excluded, and leaving 46 articles in hand. A more in-depth review of the literature excluded another 10 and we ended up with 36 articles.

DISCUSSION

The gut microbiota

Gut microbiota is microorganisms that inhibit the gut and have a symbiotic relationship with the host.[3,6] The human gastrointestinal tract consists of almost 100 trillion microorganisms which are varied in species. As the oxygen level is decreased, the density of bacterial cells in the gastrointestinal tract is progressively increased and it may reach 10^{11} to 10^{12}/mL in the colon.[6,9] Some phylums which predominant in the human intestine are Firmicutes, Bacteroidetes, Actinobacteria,

Proteobacteria, Fusobacteria, and Verrucomicrobia. Bacteroidetes (*Bacteroides, Provetella,* and *Xylanibacter*) and Firmicutes (*Ruminococcus, Clostridium, Lactobacillus, Eubacterium, Faecalibacterium,* and *Reseburia*) are the two most abundant phyla that makeup 90% of the microbiomes.[3,9]

The gut microbiota confers roles in host health, such as protecting the gastrointestinal tract by adding additional metabolism pathways for certain substances (e.g. vitamins), energy production, and improving the immune system. It also contributes to the biotransformation of conjugated bile acids.[3,10] Some physiological effects of gut microbiota are presented in TABLE 1.

The distal gut is a supportive environment for bacterial growth due to its high molecule concentration of nutrition for microbiota growth.[3] The abundance of microbiota in each individual is particularly determined by the type of food consumed.[6,9] Some carbohydrates and proteins which are not digested by some proximal gut are anaerobically metabolized by bacteria which is known as fermentation. Various fermentation products give different effects for each individual. Carbohydrate is an important nutrition in colonic microbe metabolism to produce energy and other end products such as methanes, hydrogens, and short-chain fatty acid (SCFA).[3,6]

When the requirement of indigestible carbohydrate is not met, protein is used for bacterial growth and this condition negatively impact the growth of saccharolytic bacteria. In turn, the protein will be fermented by the proteolytic bacteria, such as *Clostridium* and *Bacteroides*, to produce energy by the deamination reaction. Nevertheless, this pathway produces potentially toxic metabolites (such as ammonia, amines, phenols, and indoles), which are excreted in the stool and the healthy kidney.[3,11]

The ratio of carbohydrates to proteins is essential for nutrition. This ratio will be reduced in the distal gut since carbohydrate is well fermented by gut distal microbiota. Individuals with the problem of slow gut transit (such as constipation) will have more growth of proteolytic bacteria, which can contribute to producing metabolic toxins and induce pro-inflammatory cytokines.[3] Some individual factors, including gut acidity, antibiotics consumption, nutrient intake, psychological and physical stress, bowel wall edema, iron intake, genetics, and other diseases beyond the gastrointestinal problems could affect the gut microbiota balances, known as gut dysbiosis. These factors may further affect the overgrowth of the pathogenic bacteria, that can be translocated to the bloodstream.[12-4]

In healthy individuals, the gut barrier, including tight junction, enterocyte membranes, mucus, and gut wall immune system acts as a defense mechanism to prevent gut translocation, for instance, lumen materials or gut microbiota.[15] The tight junction complex binds to the epithelial cells to prevent paracellular translocation. The tight junction consists of adhesive proteins such as occludin, claudin (the primary protective protein from solute and fluid diffusion), zonula occludent (ZO) protein, a cytosol protein, and actin and myosin peri junctional ring that regulate the paracellular permeability.[3,15] The tight junction regulates its tightness based on physiological needs. It is the most efficient barrier against microbes, lipopolysaccharides, toxic fermentation products, digestive enzymes, and other potentially dangerous substances that can be translocated from the gut to the whole body. The gut immune system is essential in maintaining the dynamic balance between gut microbiota and host symbiosis. A balanced immune system is needed to preserve gut homeostasis, involving the good interaction between adaptive immunity, IgA, and T-cell regulator.[3,15-17]

Table 1. Physiological effects of the gut microbiota.[3]

A. Gastrointestinal tract integrity and function
1. Tight junction protein structure restoration
2. Induction of epithelial heat-shock protein
3. Upregulation of mucin genes
4. Compete with the pathogen bacteria in binding with the gut epithelial cells
5. Secrete antimicrobial peptide
6. Suppress the gut inflammation
B. Effects in immune systems
1. Maturation of gut immune system
2. Decrease allergic response to food and environmental antigens
3. Increase immunomodulation and cell differentiation
C. Metabolic effects
1. Destroy indigestible polysaccharides
2. Help the absorption of complex carbohydrate
3. Vitamin K synthesis
4. Amino acids synthesis (threonine and lysine)
5. Biotransformation of conjugated bile acid
6. Food oxalate degradation

Gut dysbiosis in CKD

From the early stage of CKD, the gut microbiota changes quantitatively and qualitatively in its composition and metabolic activities. These changes may be due to the alteration in food transit time, lower protein absorption, decreased fiber intake, oral iron supplementation, and frequent antibiotic usage.[3,6] Many factors affect CKD patients' longer colonic transit time, such as the dialysis modality, lifestyle, physical inactivity of the patient, phosphate binders, food restriction, fluid intake limitation, and other comorbid diseases such as diabetes, heart failure, and stroke. CKD patients are usually advised to restrict potassium intake, which means less intake of fruit and vegetables, which may further decrease fiber intake.[3,18] In the uremic state, the digestion and the absorption of protein are disturbed and may increase the protein entering the colon, which consecutively is degraded by the proteolytic bacteria.[3,19] Antibiotic usage may also change the colonic microbiota.[3] All these factors can contribute to inducing systemic inflammation and accumulate the uremic toxin, which is possible to translocate from the gut and excreted by the kidney. Inflammation and the uremic toxin play central roles in atherosclerosis and other complications of CKD.[6,20]

Alteration in the gut barrier of CKD patients can lead to increase gut permeability. In uremia, gut barrier dysfunction is often found and manifested as endotoxemia without clinical infection. This is caused by the alteration of gut microbiota. Elevation of urea level and urease-producing bacteria will increase the ammonia in the gut lumen. This condition changes gut acidity and increases gut permeability by disrupting the tight junction of the enterocytes.[6,15] Vaziri *et al.*[15] showed a significant decrease of tight junction protein, claudin-1, occludin, and ZO-1, in the colon mucosal layer of CKD patients. It was related to the infiltration of mononuclear cells in

the lamina propria and increased the thickness of the colonic mucosa.[15]

CKD patients often experience edema and hypervolemia affecting the gut barrier dysfunction, especially ESKD patients who are on hemodialysis or peritoneal dialysis. In contrast, excessive ultrafiltration and hypotension during the hemodialysis will result in transient gut ischemia, and increases gut permeability and endotoxin translocation.[15]

Decreased pro-inflammatory cytokine clearance in CKD is related to oxidative stress and inflammation development. Inflammation is the most significant factor in the disease progression and CKD complications, such as cardiovascular disease, cachexia, and anemia. Oxidative stress and chronic inflammation stimulate NF-κB, a transcription factor that regulates pro-inflammatory cytokines and chemokines. Increased gut barrier permeability in CKD may translocate bacterial products from the gut, which is proven by the DNA fragments of gut pathogens (both aerobic and anaerobic) in the circulation, whether they are at an earlier stage of CKD or on renal replacement therapy. These circulating bacterial products activate the innate immunity and induce CKD-related inflammation, which further increases cardiovascular disease and mortality.[6,12,21]

Gut microbiota products are one of the important factors that can cause uremic toxin in CKD patients. The gut bacteria microbiota degrades almost 10 grams of protein to produce ammonia, thiol, phenol, and indole. Some fermentation products are excreted in stool, while others are absorbed and excreted by the kidney. These products are found and accumulated in CKD patients. Phenols (p-cresol, p-cresyl sulfate (PCS), and p-cresyl glucuronide) and indole (indoxyl sulfate/IS) are the uremic toxins produced by the gut microbiota, which are bound to protein. P-cresol or PCS is the product of phenylalanine and tyrosine fermentation, while IS is the product of tryptophan fermentation.[3,6,19,22]

The uremic toxin gives biological effects that can disturb other tissues. Both IS and PCS are related to fibrosis, decreased renal function, and disease progression. Indoxyl sulfate is associated with endothelial injury, arterial stiffness, aorta calcification, profibrotic effects in the heart, cardiomyocyte hypertrophy, and predisposition factor of atrial fibrillation. In CKD patients with hemodialysis, PCS and IS are also related to peripheral vascular disease and venous access thrombosis.[3,23-25] In a meta-analysis study reported that increased PCS and IS were associated with increased mortality in CKD patients, and increased PCS was also associated with an increased risk of cardiovascular events.[26]

Anemia in CKD is related to the IS, which interferes with erythropoietin and induces the apoptosis of erythrocytes.[27,28] Indoxyl Sulphate also decreases bone formation. It increases oxidative stress in the osteoblast, and further increases the resistance of parathyroid hormone, leading to the adynamic bone. There is a correlation between FGF-23 serum and IS. It marks the relationship between this molecule and metabolic bone disease in uremic patients.[6,29,30]

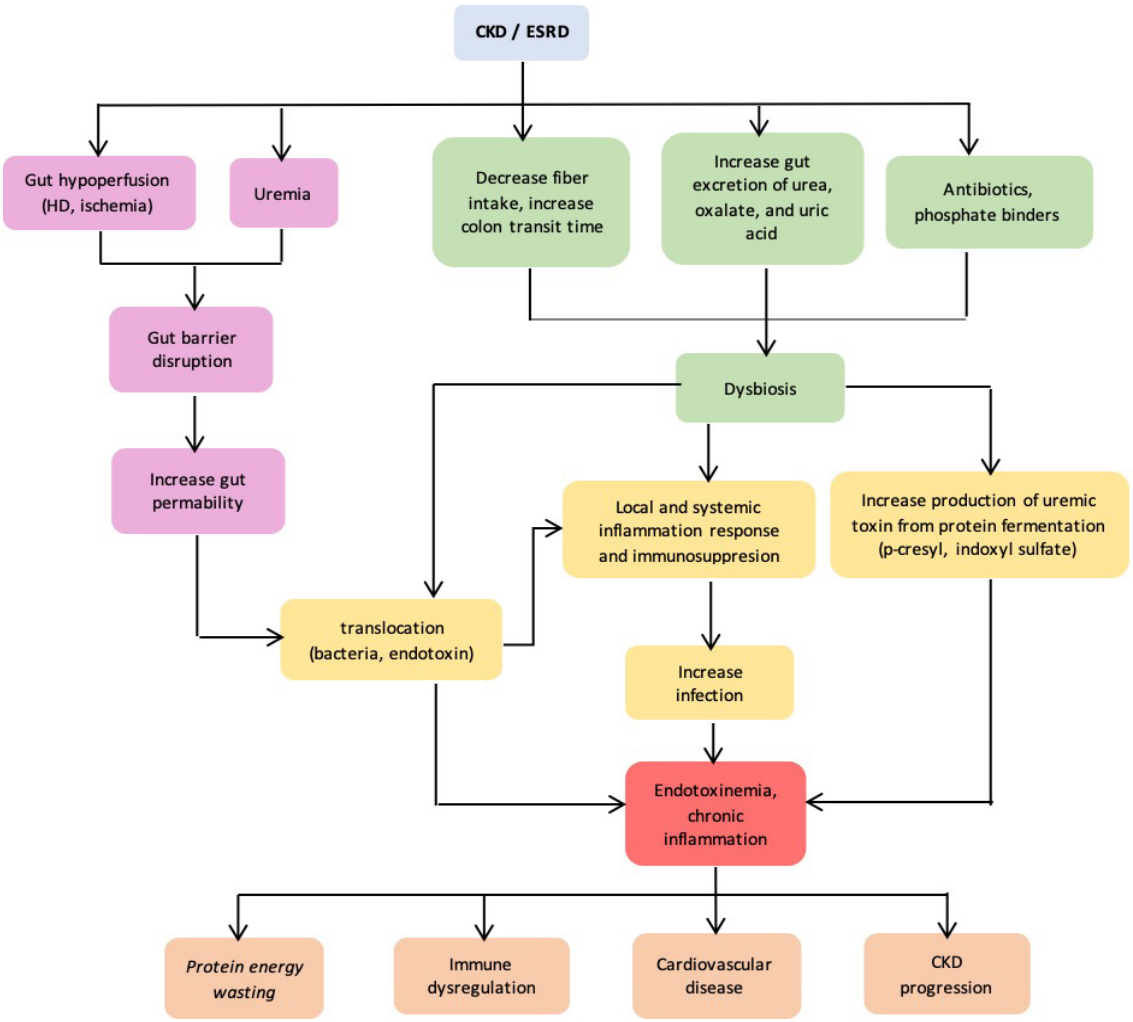

FIGURE 1. Relationship between uremia in CKD/ESKD on the gut barrier and microbiota. (Reproduced with permission from Sabatino, *et al.*)[3]

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Probiotic role in CKD

Several ways have been used to decrease uremic toxin, oxidative stress, and inflammation in CKD patients, including the restoration of gut microbiota balance. High-fibre diet increases the production of SCFA, the energy source of the gut microbiota, and it plays an important role in maintaining the function and integrity of gut mucosa. Fibre is used to lessen gut transit time, decreasing the time for amino acid fermentation. A low ratio of protein and fiber in the diet will bring benefits for CKD patients since the ratio correlates with the PCS and IS levels.[31,32] A deficient protein diet (0.3 grams/kg body weight/ day) combined with amino acid keto-analogue will decrease IS levels in CKD patients.[33,34]

Uremic toxin production can be lowered by increasing saccharolytic bacteria which digest the fiber and decreasing proteolytic bacteria in the colon. Probiotic supplementation is given in CKD to decrease or excrete the uremic toxin by reducing the conversion of amino acid to PCS and IS. Probiotic is live microorganism which when administered in adequate amounts confer a health benefit on the host.[12,35] The microorganism is modified genetically to produce some specific exogenous enzyme which can stand stomach acid and bile salt.[12,35,36] It increases epithelial cell integrity. For instance, probiotics can inhibit the pathogen entry to epithelial cells and create a physical barrier and mucus by increasing the synthesis and secretion of mucin from goblet cells. Probiotics also protect gut integrity by inducing ZO-1 expression that can increase the intercellular tight junction between the gut epithelial cells.[35,36]

Probiotics also decrease gut infection, especially the risk of Clostridium difficile infection in CKD patients. Some probiotic strains produce antibacterial substances, called bacteriocin or antimicrobial peptides. *Lactobacillus* is capable of producing lactic acid that has an antimicrobial effect by reducing the local gut acidity.[35] Moreover, *Lactobacillus* also can induce and increase innate and adaptive immunity. Some probiotic strains help B cells differentiation and increase IgA production. Other strains stimulate the innate immune system by stimulating the dendritic cells, which then go to the mesenteric lymph nodes, inducing the T regulatory cell and producing the anti-inflammatory cytokines (IL-10 and TGF-b).[35] Wang *et al.*[37] conducted a study in ESKD patients with peritoneal dialysis who were supplemented by probiotics for 6 mo. It was found a significant decrease in endotoxin and pro-inflammatory cytokines (TNF-a and IL-6), and increased IL-10 with preservation of residual renal function.

Another study by Jia *et al.*[38] reported that probiotic supplementation decreased PCS in CKD and increased IL-6 levels. But, it did not affect serum creatinine, BUN, and hemoglobin levels. The increased level of IL-6 after probiotics supplementation is influenced by other factors, including the probiotic strain. IL-6 may act as pro- and anti-inflammatory cytokines, which is related to the signal transducers and activators of transcription (STAT) 1 and STAT 3 that activates and suppresses NF-κB activity.[38,39]

A study by Fagundes *et al.*[40] reported the health benefits of *Lactobacillus* and *Bifidobacterium* supplementation in CKD patients. They found a decrease in urea, BUN ammonia, plasma level of PCS, and IS in the group with probiotics supplementation. In addition, probiotics supplementation can increase the absolute number of *Bifidobacteria* population, both native and supplemented, which is beneficial in gut mucosal barrier function, decrease cytokine and endotoxin, and increase IL-10.[40,41]

CONCLUSION

Gut dysbiosis leads to the elevation and the accumulation of absorbed uremic toxins, and later increases oxidative stress and chronic inflammation in CKD patients. Moreover, an increase in gut barrier permeability in CKD also elevates the amount of circulating endotoxin and promotes inflammation. Specific strain probiotic supplementation is an alternative that benefits CKD patients by slowing the disease progression and decreasing chronic inflammation.

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Moringa oleifera Lam. to accelerate wound healing: a review

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ABSTRACT

An injury to the skin that disrupts the soft tissue may form a wound. The healing process in response to injury is a dynamic and well-regulated process of cellular, humoral, and molecular mechanisms that consists of four partly overlapping phases: hemostasis, inflammation, proliferation, and remodeling. An impaired wound-healing process may cause a formation of an abnormal scar and chronic wounds, leading to a reduced life quality. Therefore, it needs an optimal prevention strategy. Many modalities have been claimed to accelerate wound healing. The trend of using natural products is increasing in most Southeast Asian countries due to their biodiversity. Nowadays, studies on natural compounds are increasing to accelerate wound healing. *Moringa oleifera* Lam. is a high-value plant that each part of it has a high nutritional value as well as a great range of medicinal uses, including anti-inflammatory, antimicrobial, antioxidant, and wound healing properties. In this review, we have explored the *M. oleifera* that are very rich in vitamins, minerals, fatty acids, and phytochemical compounds like quercetin, kaempferol, and vicenin-2, that play a role in the wound healing process. Moreover, these compounds may enhance the healing of wounds with pathological conditions such as diabetes, immunocompromised and persistent infection.

ABSTRAK

Cedera pada kulit yang mengganggu jaringan lunak dapat membentuk luka. Proses penyembuhan sebagai respons terhadap cedera adalah proses yang dinamis dan diatur dengan baik dari mekanisme seluler, humoral, dan molekuler yang terdiri dari empat fase yang saling tumpang tindih: hemostasis, inflamasi, proliferasi, dan remodeling. Gangguan pada proses penyembuhan luka dapat menyebabkan pembentukan jaringan parut abnormal dan luka kronis, yang menyebabkan penurunan kualitas hidup. Oleh karena itu, diperlukan strategi pencegahan yang optimal. Banyak modalitas telah diklaim dapat mempercepat penyembuhan luka. Tren penggunaan produk alami meningkat di sebagian besar negara Asia Tenggara karena keanekaragaman hayatinya. Saat ini, penelitian tentang senyawa alami untuk mempercepat penyembuhan luka semakin meningkat. *Moringa oleifera* Lam. adalah tanaman bernilai tinggi yang memiliki nilai gizi tinggi serta berbagai kegunaan obat, termasuk sifat anti inflamasi, antimikroba, antioksidan, dan untuk penyembuhan luka. Dalam ulasan ini, kami telah mengeksplorasi *M. oleifera* yang sangat kaya akan vitamin, mineral, asam lemak dan senyawa fitokimia seperti kuersetin, kaempferol, dan vicenin-2, yang berperan dalam proses penyembuhan luka. Selain itu, senyawa ini dapat meningkatkan penyembuhan luka dengan kondisi patologi seperti diabetes, imunokompromis dan infeksi persisten.

Keywords:
Moringa oleifera Lim.; pathological wound; phytochemical; wound healing; biological activity

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INTRODUCTION

Skin is the multi-function and the largest organ in the human body. One of those functions is the first-line protector against pathogens, toxins, and trauma. If trauma or injury happens, the skin also play role in the wound healing mechanism. If the integrity of the skin is damaged or loss caused by injury or disease, it could lead to morbidity or even death.[1]The Process of wound healing is very dynamic and complex, as a response to injury, cells in the skin will be activated to promote wound healing, which also attracts many other cells and substances from other parts of the body.[2]

The abnormalities of wound healing refer to wound scars or chronic wounds. Lots of patients develop wound scars after burns, trauma, or surgery annually worldwide. Patients with scars were reported to have decreased quality of life, physical status, and psychological health.[3] Delayed chronic wound healing is one of the major biomedical and economic burdens for the global healthcare system.[4] Nowadays, healing wound healing is still a challenging clinical problem. To have proper and efficient management in wound is crucial.[5]

Currently, existing wound management such as antimicrobial agents, modern dressings, maggots, surgical treatment, tissue sealant & platelet gel, skin substitutes, cytokines & growth factors, and recombinant hormones/enzymes cost a lot of money and are not yet available in some developing countries.[6,7] So that currently, the majority of the trend is back to using herbal products in addition to adopting a more natural way of life.

One of the most biodiverse regions on Earth is Southeast Asia region. Even though the region occupies just a small percent of the Earth's surface, it has the highest global diversity for plant, animal, and marine species.[8] The trend of using natural products is increasing in most Southeast Asian countries.[9] According to the National Agency of Drug and Food Control (NADFC) of Republic of Indonesia, many natural substances have been registered for their medical use officially; the bigger remainder is used traditionally.[10]

Moringa oleifera is a great value plant that grows in many tropical and subtropical regions. *Moringa oleifera* is usually known as 'horseradish tree' or 'drumstick tree'. *Moringa oleifera* is also known in various regions in Indonesia under different names such as kelor (Java, Sunda, Bali, Lampung), maronggih (Madura), moltong (Flores), keloro (Bugis), ongge (Bima), and hau fo (Timur).[11] *Moringa oleifera* is a high-value plant that each part of it has high nutritional value and a great range of medicinal uses.[12,13] The empirical use of *M. oleifera* in Indonesia is as food, cosmetics, and medicine. It is used both orally or topically.[14,15] *Moringa oleifera* is very rich in vitamins, minerals, fatty acids, and phytochemical compounds like quercetin, kaempferol, and vicenin-2.[16,17] These compounds are trusted for anti-inflammatory, antimicrobial, antioxidant, and wound healing properties. This article discussed how *M. oleifera* may accelerate the healing of a wound in pathological conditions such as diabetes, immunocompromised and persistent infections.

MATERIAL AND METHODS

We queried PubMed, Semantic Scholar, Google Scholar, and ScienceDirect database. We included the following search terms: "*Moringa oleifera*", "*Moringa oleifera* leaves", "*Moringa oleifera* seeds", "*Moringa oleifera* bark", "wound healing", "abnormal wound healing", "chronic wound." The reference lists of the included articles and the relevant links were also manually reviewed

for additional eligible articles. Studies published in English with full text available were included. The inclusion criteria for this narrative review included the studies regarding the use of *M. oleifera* for wound healing and chronic wound healing.

RESULTS

A total 49.707 articles were found based on a search on the database according to keywords. The articles were screened to evaluate duplication and then reanalyzed to ensure eligibility according to the predetermined inclusion criteria i.e. "*M. oleifera*" and "wound healing" and "chronic wound healing". A total 61 journals that met the inclusion criteria were then reviewed.

DISCUSSION

Wound healing

The wound-healing process is a dynamic and well-regulated process of cellular, humoral, and molecular mechanisms that begins immediately after injury and may last for years.[2] The process consists of four partly overlapping phases, which are hemostasis, inflammation, proliferation, and remodeling.[18]

Hemostasis phase

The wound will cause blood leakage from damaged blood vessels and results in rapid recruitment of platelets leading to clot formation, which next acts as a temporary shield that protect the bare blood vessels from more leakage and prevent the entry of pathogens.[19] This first wound-healing phase begins immediately after wounding.[20] Platelets will be activated so that they will be degranulated and released chemotactic and growth factors, like platelet-derived growth factor (PDGF), transforming growth factor-ß (TGF-ß), epidermal growth factor (EGF), insulin-like growth factors (IGF), proteases, and vasoactive agents (serotonin, histamine). Platelet activation causes chemokines release lead to attracting inflammatory cells to the area and initiate the next phase of the healing process.[21]

Inflammation phase

The early period of vasoconstriction is usually just 10-15 min in duration and is followed by a more persistent vasodilation period that is mediated by histamine, prostaglandins, kinins, and leukotrienes. Within hours of injury, the inflammatory phase cellular aspect occurs, and it includes mast cells, macrophages, neutrophils, and lymphocytes.[21]

Immediately after injury, mast cells become activated, degranulate, and release a large number of mediators like inflammatory cytokines, vascular permeability factors, vasodilation agents, and proteases which increases the recruitment of immune cells to the site of injury.[22] Damage-associated molecular patterns (DAMPs), lipid mediators, hydrogen peroxide (H_2O_2), and chemokines released by injured cells also provide signals for inflammatory cell recruitment, particularly neutrophils.[23] Neutrophils release cytokines like TNF-α, IL-6, and IL-1β, which intensify the inflammatory response and stimulate VEGF and IL-8 for an adequate repair response.[2]

The wound induces macrophages accumulation in the first 24 – 48 hours at the site of injury. In the wound healing early stages, macrophages are pro-inflammatory and microbicidal, expressing TNF-α, IL-6, and IL-1β.[23] Macrophages are also responsible for clearing and inducing apoptotic cells (including neutrophils), thus paving the way for inflammation resolution. As macrophages clear these apoptotic

cells, they go through a phenotypic transition to a regenerative state that stimulates keratinocytes, fibroblasts, and angiogenesis to promote the regeneration of tissue. In this way, the transition to a proliferative healing phase is promoted by macrophages.[20]

Proliferation phase

In the proliferation phase (about three to ten days after injury) the healing process's main focus is on the wound surface closure, granulation tissue formation, and vascular tissue restoration. Because of that, besides local immigration of fibroblasts along the fibrin tissue and the initiation of re-epithelialization from the wound margins, angiogenesis, and neovascularization are enabled by the growing capillaries.[2,24]

At the end of the inflammatory phase, angiogenesis occurs. Angiogenesis involves proliferation, migration, and branching of endothelial cell to form new blood vessels. While new blood vessels arise, resident fibroblasts proliferate and invade the clot to form contractile granulation tissue. The dividing fibroblasts store the ECM and shift the microenvironment of a wound from an inflammatory state to a regenerative state. In this phase, many fibroblasts differentiate into myofibroblasts, retracting the wound margins together.[23] Myofibroblasts are known to play a central role in sealing wound tissue, through their capability to generate strong contractile forces.[25] About four days after wounding, myofibroblasts appear in the wound. Myofibroblasts exert their contractile forces through focal adhesion contacts that connect the intracellular cytoskeleton to the ECM.[26]

Remodeling phase

As the wound healing final phase, the remodeling phase is responsible for new epithelium development and the formation of mature scar tissue. The remodeling phase begins two to three weeks after the onset of injury and can last up to one to two years, or sometimes for a longer period of time. The remodeling phase aims are reorganizing and maintaining a balance between degradation and synthesis, leading to the healing of the wound with a "normal" tissue structure. The final wound strength obtained depends on the localization of the repair and its duration, but the tissue's original strength may never be regained.[5,27]

The remodeling phase consists of neovascularization regression and periodic deposition into the ECM and reconstitution of granulation tissue into scar tissue. Granulation tissue mainly consists of collagen type III, which is partially replaced by the stronger collagen type I as wound remodeling progresses.[23] As the wound heals, the density of fibroblasts and macrophages is progressively reduced by apoptosis. Over time, capillary growth stops, blood flow to the area decreases and metabolic activity at the wound site decreases. The final result is the scar that fully matured with reduced cell and blood vessel numbers and a high tensile strength tissue.[5]

TABLE 1. Physiological wound healing phases[28]

Wound healing phase	Histological event changes	Duration
Hemostasis	Vasoconstriction, platelet aggregation, clot formation, vasoactive agents, glycoprotein, coagulation	Minutes to hours
Inflammation	Vasodilation, neutrophils, macrophages, lymphocytes, mast cells, chemokines, growth factors, interleukins	Hours to days
Proliferation	Fibroblasts, collagen type III, granulation tissue, reepithelization, keratinocytes, growth factors, cytokines, neovascularization, endothelial cells, fibronectin, glycosaminoglycans, proteoglycans, hyaluronic acid, myofibroblasts, α-smooth muscle actin, contraction	Days to weeks
Remodeling	Neovascularization regression, reorganization of extracellular matrix, collagen fibril crosslinking, collagen type III lysis, collagen type I synthesis, apoptosis, decreased number of cells, tensile strength, scar maturation	Weeks to years

Abnormal wound healing

The natural consequence of large or deep wounds in adult mammals is the formation of scars. There is a scar formation spectrum, with scarless regeneration on one end, "normal" scar formation in the center, and pathological formation of scar, including hypertrophic scar and keloid, on the other end. Scar formation is determined by the proliferative and remodeling phase of wound healing.[29] Abnormal scar formation is exacerbated when the inflammation response is excessive.[30] The anti-inflammatory cytokine IL-10 decreases scarring, but the pro-inflammatory cytokines IL-6 and IL-8 have the opposite effect on the scar tissue response. Superficial injuries that do not reach the reticular dermis never cause keloids and hypertrophic scars. This suggests that pathological scars are caused by wounds in this skin layer and aberrant wound healing within them, which is characterized by persistent inflammation and histologically localized.[31] A scar can have disturbing physical, aesthetic, functional, psychological, and social status so modalities are needed for prevention.[32]

In addition to scar formation, when wound healing does not proceed normally, chronic wounds will develop. These chronic wounds can be caused by underlying pathological conditions such as diabetes, abnormal immune function (immunosuppressive drugs), and persistent infections.[33] Diabetes delays the healing process because it impairs each phase of wound healing i.e. haemostasis, inflammation, proliferation, and remodeling phase. Diabetic wounds exhibit a persistent inflammatory phase associated with an impediment in the formation of mature granulation tissue and a reduction in wound tensile strength.[34] High blood sugar causes sustained production of pro-inflammatory cytokines, impaired macrophage and neutrophil function, imbalance in extracellular matrix regulation, impaired keratinocytes and fibroblast migration and proliferation, and impaired production of healing-associated factors like impaired growth factor production. In addition, high blood sugar levels cause a decrease in nitric oxide synthetase activity so that nitric oxide production decreases as a result, reactive oxygen species increases affect vasoconstriction, impaire platelet function and angiogenesis, and prolong inflammation.[34,35] It takes a modality that can overcome these.

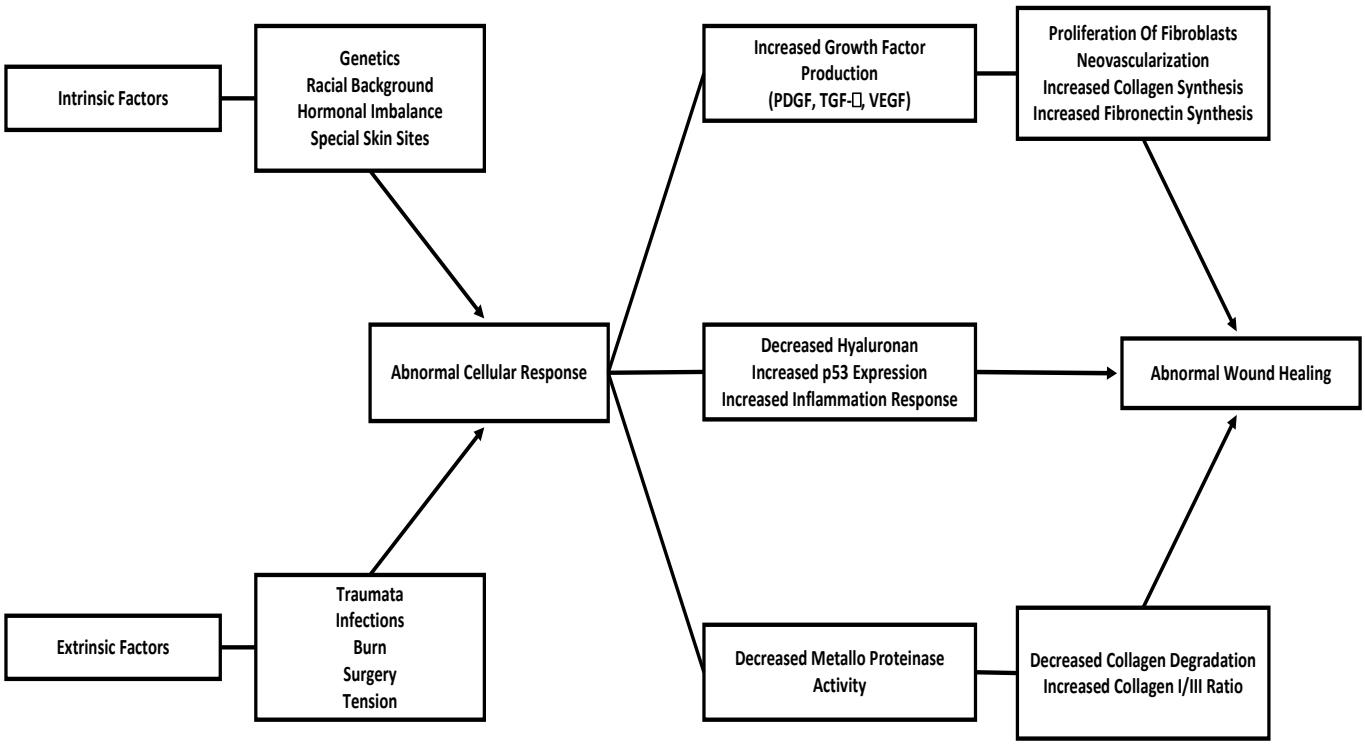

FIGURE 1. Pathogenesis of abnormal wound healing[36]

Moringa oleifera compounds in promoting wound healing process

Moringa oleifera is a cruciferous plant that belongs to the Moringaceae family. It is usually named horseradish tree or thigh tree by locals and is popular as a staple food in many parts of the world. *Moringa oleifera* is consumed not only for its high nutritional value but also for its abundant medical benefits. It is rich in proteins, minerals, β-carotene, ascorbic acid, tocopherol, polyphenols, and flavonoids which serve as a good source of natural antioxidants. Recently, it was reported to enhance various biological functions including anti-inflammatory, antimicrobial, and wound-healing properties.[37,38]

Methanol and aqueous extracts of roots and bark, methanolic extract of leaves and flowers, and ethanolic seeds extract of *M. oleifera* have anti-inflammatory activity. *In vitro* studies of hot water infusion of flowers, leaves, roots, seeds, and stems or bark of *Moringa* show anti-inflammatory activity. *Moringa oleifera* leaves, roots, bark, and seeds also exhibit antimicrobial activity against bacteria and fungi.[39]

Rate of wound healing in abrasion and excision wounds in *in vivo* study was highly increased with 0.5% *M. oleifera* leaves aqueous extract film dressing. This can be related to (1) the rapid release of bioactive compounds in a 0.5% the aqueous extract film dressing to promote wound contraction (increase collagen deposition and composition by increasing COL1α1 expression), (2) the reduction of inflammatory phase after the rapid transition to the process of epidermal regeneration (upregulation of VEGF, IL-6, TNF-α) and (3) its ability to form a protective gel on the bed of wound against damage from external after absorption of exudate.[40]

Moringa oleifera leaves extract per oral (gavage) has contributed to a glycemic reduction in diabetic rats lead to improv the wound-healing process.[41] *Moringa oleifera* -treated

wounds showed a significant increase in fibroblast growth and proliferation, as well as faster fibroblasts migration in rats. Furthermore, the wounds treated with *M. oleifera* extract showed increased new collagen formation and collagen deposition into the wound area.[42] The *M. oleifera* is known to contain phytochemical compounds such as flavonoids, tannins, saponins, and other phenolic compounds that have antimicrobial activity so that they can play a role in accelerating wound healing. Gothai *et al.*[43] reported that *M. oleifera* ethyl acetate (EtOAc) fraction at the 12.5 and 25 μg/mL concentration promotes cell proliferation and migration of normal human dermal fibroblasts.

Bioactive compounds of *M. oleifera* extract that may enhance wound healing are quercetin and kaempferol in the crude extract of methanol, and the flavone compound C-glycoside vicenin-2 in the aqueous fraction. Quercetin, vicenin-2, and kaempferol were detected in the aqueous extract of *M. oleifera* leaves to have an anti-inflammatory effect in an *in vitro* study so which may accelerate wound healing.[44] Vicenin-2 (VCN-2) is a bioactive compound and flavonoid glycoside from a subgroup of phenolics which is known as apigenin-6,8-di-C-β-d-glucopyranoside. It has been reported to possess prospective antidiabetic, antioxidant, and anti-inflammatory properties and enhance cell proliferation and migration effect.[45]

Flavonoids are able to increase the growth factors needed for the process of wound healing, like EGF, TGF-α, TGF-β, PDGF, VEGF, and FGF so have faster wound healing. These benefits can accelerate the transition from the inflammatory phase to the proliferative phase so that wound healing is faster than the physiological process.[16] Muhammad *et al.* stated that vicenin-2, the bioactive compound in *M. oleifera* leaves which responsible for the potent effects of cell proliferation and migration. They also reported that *M. oleifera* leaves extract which contains vicenin-2 stimulates tissue cell proliferation, thereby reducing wound size in *in vivo* study. This extract also caused the activity of inflammatory mediators TNF-α, IL-1β, IL-6, iNOS, and COX-2 decreased and angiogenesis activity increased thereby reducing the time required for the process of wound healing.[17] Vicenin-2 reduces the expression of anti-HIF1α and MMP proteins so it is very important for tissue granulation, angiogenesis formation, and re-epithelialization during the process of wound healing.[46] Tan *et al.*[45] demonstrated that both TGF-β and VEGF expression of Human Dermal Fibroblast cells was enhanced in response to treatment with vicenin-2. During the process of wound healing, VEGF induces angiogenesis via endotel proliferation and migration while TGF-β triggers remodeling and fibrogenesis by development and differentiation.[45] In the presence of flavonoids, the expression of biomarkers such as TGF-β and VEGF is increased which in turn accelerates wound healing.[47]

Quercetin is a kind of polyhydroxy flavonoid, which is frequently found in flowers, leaves, and fruits of various plants. Quercetin inhibits inflammatory reactions via modulating macrophage polarization switching from M1 to M2 phenotype so that prolong inflammatory doesn't occur.[48] Quercetin has pharmacological effects including antioxidant, anti-inflammatory, angiogenic, antibacterial, immunomodulatory, increased myofibroblast activity, and proliferation of epithelial cells and fibroblasts. These properties make quercetin a promised wound-healing agent. Treatment with quercetin accelerates wound healing through (i) rapid wound contraction, (ii) controlled modulation of pro-inflammatory cytokines (TNF-α) and anti-inflammatory (IL-10), (iii) increased neovascularization via increased VEGF

and TGF- β expression, (iv) increased antioxidant status at the wound site, and (v) increased fibroblast proliferation with marked collagen deposition and increased myofibroblast formation. Expression of growth factors involved in angiogenesis, collagen synthesis, and extracellular matrix (ECM) such as VEGF and TGF-β1 increased on days 3 and 7, respectively.[49] Furthermore, it has provided a mechanism that enhances wound healing; open excision wounds in adult mice given topical 0.1% quercetin (*in vivo* study) showed accelerated wound closure results in treated mice, whereas the level of TNF-α decreased with better re-epithelization, more regular deposition of collagen, and VEGF and TGF-β1 upregulated.[50] The active compound, kaempferol and its glycosides promote wound healing through keratinocyte cell migration. Effects on keratinocyte migration of kaempferol via FAK/Akt activation and enhances cellular filopodia and lamellipodia formation via activation of Rac1.[51] Quercetin and kaempferol are flavonoids that have a similar chemical structure, which is why both of compounds have similar biological activities also, and can work synergically to maintain the function of the endothelium of blood vessel for the promotion of angiogenesis and inhibits hypoxia.[52]

Moringa oleifera also contains lauric acid, myristic acid, palmitic acid, arachidonic acid, and oleic acid that triggers fibroblasts to induce various growth factors in the wound, in particular TGF-β1 and VEGF. The *M. oleifera* leaves EtOAc fraction can be a novel candidate for dermal wound healing because of the effectiveness of its antibacterial properties against various pathogens, especially skin infection-causing pathogens. Phenolic compounds in the *M. oleifera* leaves EtOAc fraction have antioxidant activity that acts as hydrogen donors or reducing agents, which improves regeneration and organization of the new tissue in wound healing.[53] *Moringa* flowers and seeds also have antioxidant and anti-inflammatory activities due to their compounds such as ascorbic acid, carotenoids, tannins, alkaloids, glycosides, flavonoids and phenolic compounds kaempferol, quercetin, and the unique combination of fatty acids in the flower extract.[38,54]

In an *in vitro* study, a combination of *M. oleifera* and *Aloe vera* containing quercetin to treat wounds, showed the highest anti-inflammatory effect and quercetin treatment with higher phenolic content was a modality with higher anti-inflammatory activity.[55] The anti-inflammatory effect through the inhibitory activity of NF-κB, inhibits the secretion of nitric oxide and pro-inflammatory markers like prostaglandin E2, TNF-α, and IL-6, at the same time induce anti-inflammatory cytokines production like IL-10 in a dose-dependent manner. Quercetin also showed antibacterial activities by inhibit of *Staphylococcus aureus*, *S. epidermidis*, and *S. pyogenes* growth. This makes it a suitable wound treatment formulation candidate.[56]

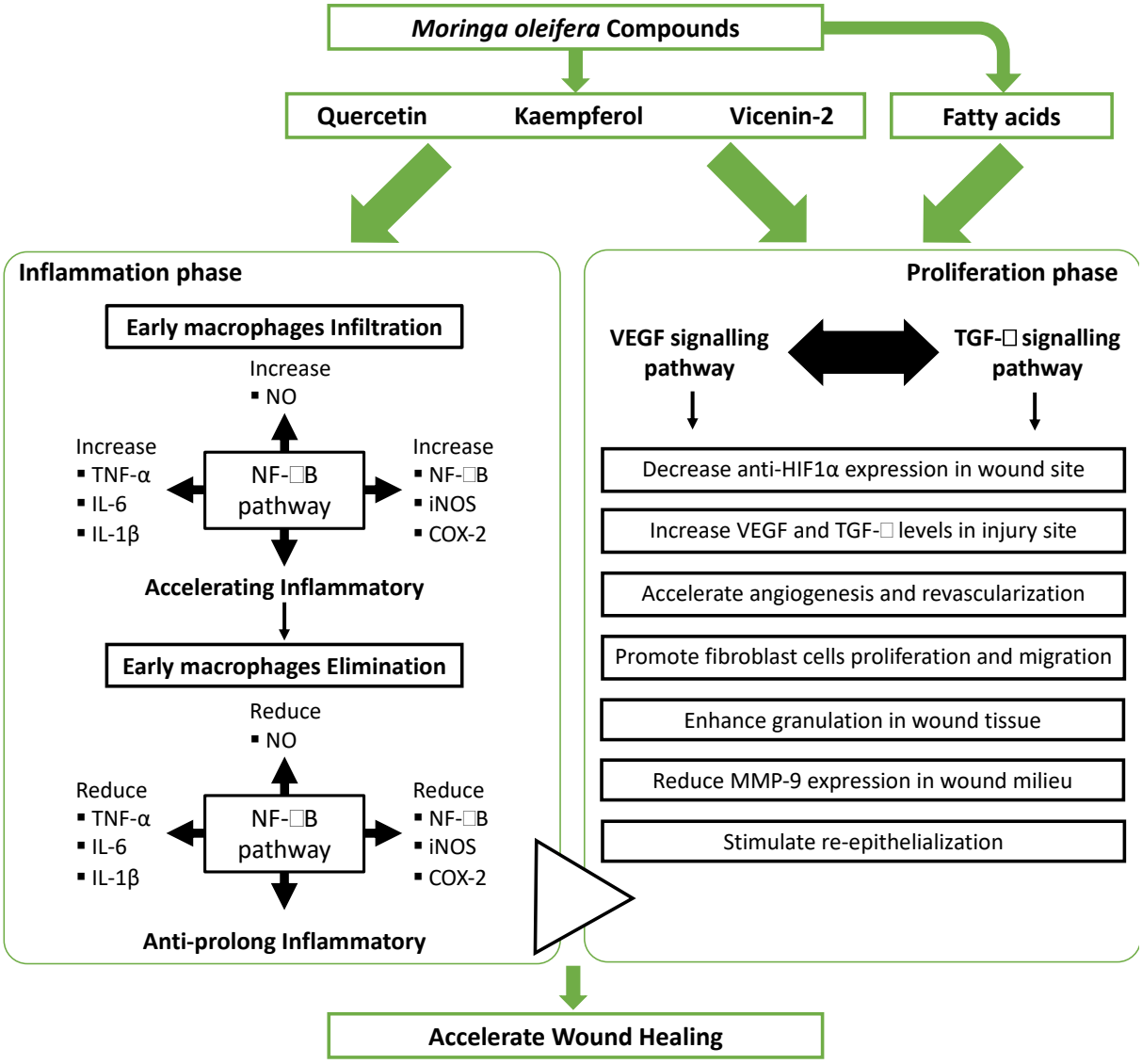

FIGURE 2. Signaling pathways of Moringa compounds to accelerate wound healing process[46]

Effect of *M. oleifera* extract on chronic wounds

Chronic wounds are associated with microorganisms due to the bacteria colonization on the wounds within 48 h after injury. These may cause an infection that affects the wound-healing process by prolonging the inflammatory phase. The antibacterial properties exhibited by an aqueous fraction of *M. oleifera* may be linked to the presence of some phytochemical compounds, such as alkaloids, triterpenoids, tannins, and flavonoids. These compounds promote the wound-healing process mainly due to their astringent and antimicrobial properties, which are responsible for wound contraction and increased rate of epithelialization. The aqueous fraction of *M. oleifera* strongly inhibiting the *S. aureus*, *P. aeruginosa*, and *E. coli* growth

suggests its ability to facilitate wound healing through antibacterial action.[16]

Topical application of aqueous fraction of *M. oleifera* reduced wound size and improved contraction rate in diabetic rats. An increase of collagen deposition and better alignment and maturation of wound tissue were observed. Downregulation of proinflammatory cytokines (IL-1β, IL-6, and TNF-α), iNOS and COX 2, and an increase of VEGF expression that promotes angiogenesis were also reported.[16] Administration of *M. oleifera* extracts on diabetic rats for 21 demonstrated significant rejuvenation of pancreatic islets, reduced serum glucose in addition to increased serum insulin level coupled with improved antioxidant status by decreasing oxidative stress/ lipid peroxidation. *Moringa oleifera* significantly suppressed levels of NF-κB in diabetic rats by enhances cellular antioxidant defense potential, therefore able to minimize abnormal cell proliferation.[57] Healing impairment in diabetic ulcers has a number of physiological causes including diminished fibroblast proliferation and angiogenesis. The *M. oleifera* ethyl acetate fraction supported the wound healing activity by promoting the proliferation and migration of fibroblast cells and neovascularization.[53]

Moringa oleifera contains amino acids, fatty acids, vitamins, and trace elements that are all important in cell-mediated immune responses. Fatty acids, vitamin D, and trace elements that not only facilitate the proliferation and maturation of neutrophils, but also the secretion of cytokines that enhance neutrophil migration and adhesion. This suggests that the presence of these compounds in *M. oleifera* extract may be useful in protecting the body. Vitamins A, C, K, and amino acids present in *M. oleifera* extract can be attributed to the ability to activate lymphocytes and their accessory cell types leading to increased antibody production in previously immunosuppressed animals thereby enhancing cell-mediated immunity. Therefore, plant extracts can be used when the immune system is compromised to enhance cell-mediated immune responses as the extracts enhance the phagocytic activity of neutrophils and increase antibody production.[58]

Moringa oleifera seeds oil and its principal compound oleic acid increased tissue collagen during the chronic wound healing process, and reverted contractile reduction of myofibroblasts in the amelioration of immunosuppression and diabetes. It also accelerates the inflammatory phase of regular injury repair, increases TNF-α concentration and neutrophil numbers in the area of injury, and reduces IL-1, IL-6, and MIP-3α concentrations. It is responsible for efficiently accelerating inflammation, thus, appropriately stimulating fibroblast activity, wound myofibroblast contraction, and matrix deposition in chronic wound healing in immunosuppressed and diabetic mice.[59] Water-soluble lectins from *M. oleifera* seeds promoted immunomodulation in human peripheral blood mononuclear cells that induced a potential wound healing profile through activation of CD8+ T lymphocytes.[60]

The aqueous extract of *M. oleifera* bark increases the amount of hydroxyproline (a direct estimate of collagen synthesis) and also opposes the action of dexamethasone to some extent on collagen synthesis, maturation, deposition, epithelialization period, and hydroxyproline content. Thus, it has the potential to counteract the anti-healing effects of steroids in patients that receive steroid therapy.[61]

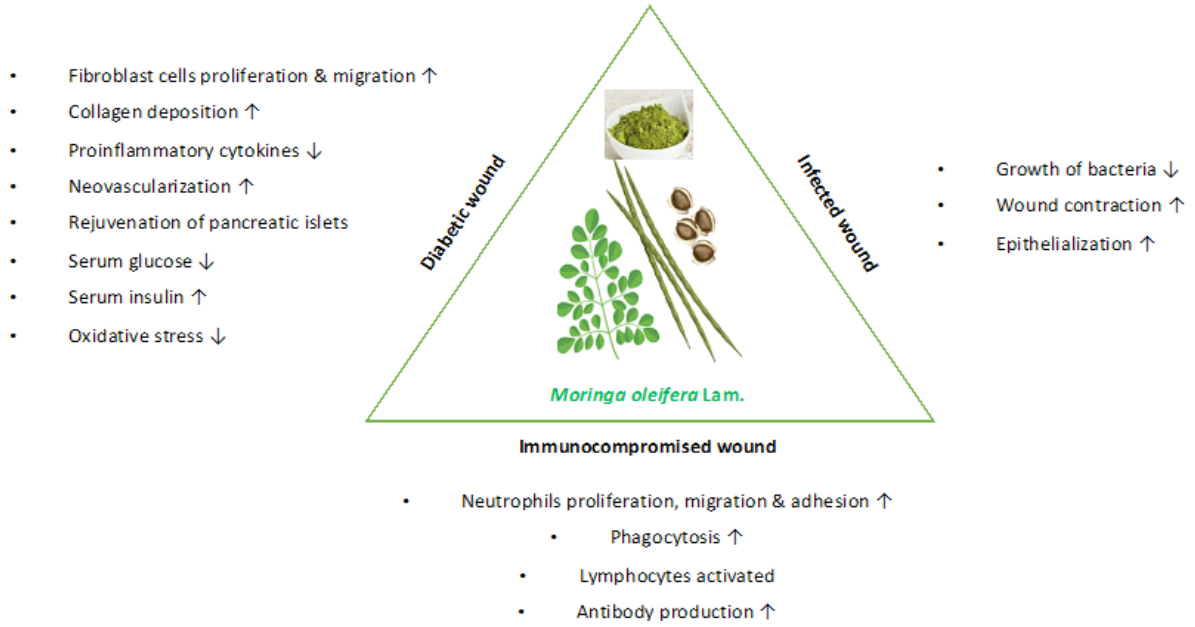

FIGURE 3. Effects of *M. oleifera* leaves in pathological wounds

CONCLUSION

In conclusion, *M. oleifera* extract which contains vitamins, proteins, minerals, fatty acids, and phytochemical compounds like quercetin, kaempferol, and vicenin-2 can accelerate wound healing by reducing the time of the inflammatory phase and the transition time to regeneration, proliferation and migration phase of epidermal due to many growth factors that contribute to cell proliferation, triggering collagen and angiogenesis formation. *Moringa oleifera* extract has activity in enhancing wound healing and it may be one of the modalities used in chronic wounds on patients with diabetes mellitus, immunocompromised and persistent infections. Further clinical studies are needed to prove the potential effects of *M. oleifera* as wound healing agents.

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Corrigendum:

Effects of 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chroman-4-one on serum levels of antioxidant enzymes in hyperlipidemic rats

(Volume 55, Number 2, 2023: Page: 99-107 https://doi.org/10.19106/JMedSci005502202301)

https://doi.org/10.19106/JMedSci005503202311

In the original article, both of authors and institution ware incorrect. The correct version is given below:

Prasetyastuti[1]*, Noviyanty Indjar Gama[2], Rahmah Dara Ayunda[3]

[1]Department of Biochemistry, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta; [2]Faculty of Pharmacy, Universitas Mulawarman, Samarinda, [3]Department of Biochemistry Faculty of Medicine Universitas Mataram Indonesia

Errors in Abstract:

Dose 10,30 and 90 mg/200g BW were higher than control group that was not intervened.

Correct version

Dose 10,30 and 90 mg/200gBW were higher than the hyperlipidemic group

Errors in Abstrak:

10,30 dan 90 mg/200g BW lebih tinggi dibanding kelompok kontrol yang tidak di intervensi

Correct version

10,30 dan 90 mg/200g BW lebih tinggi dibanding kelompok hiperlipidemia

Errors in Abstrak:

Tikus hiperglikemia dibuat dengan diinduksi makanan kaya kolesterol dan asam kolic. Enzim SOD, CAT dan GPx dianalisis menggunakan metode spektrofotometri.

Correct version

Tikus hiperglikemia dibuat dengan diinduksi makanan kaya kolesterol dan asam kolic. Perlakuan diberikan secara oral dengan disonde. Setelah 4 Minggu perlakuan darah diambil. Enzim SOD, CAT dan GPx dianalisis menggunakan metode spektrofotometri.

Ethical Clearance Number Errors on Materials and Methods

Universitas Gadjah Mada (No.KE/FK/08/8/EC/2017).

Correct version

Universitas Gadjah Mada (No.KE/FK/0818/EC/2017).

Errors in Results (Serum level of SOD)

The results showed that the cholesterol-induced rats (HL) had lower serum SOD levels than the normal group (N) (FIGURE 1). Serum SOD levels in hyperlipidemic rats that were intervened with 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chroman-4-one dose 10,30 and 90 mg/200g BW were higher than HL group that was not intervened.

Correct version

The results showed that the cholesterol-induced rats (HL) had lower serum SOD levels than the normal group (N) (FIGURE 1). Serum SOD levels in hyperlipidemic rats that were intervened with 7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-chroman-4-one dose 10,30 and 90 mg/200g BW were higher than the hyperlipidemic group.

Error in Figure 1

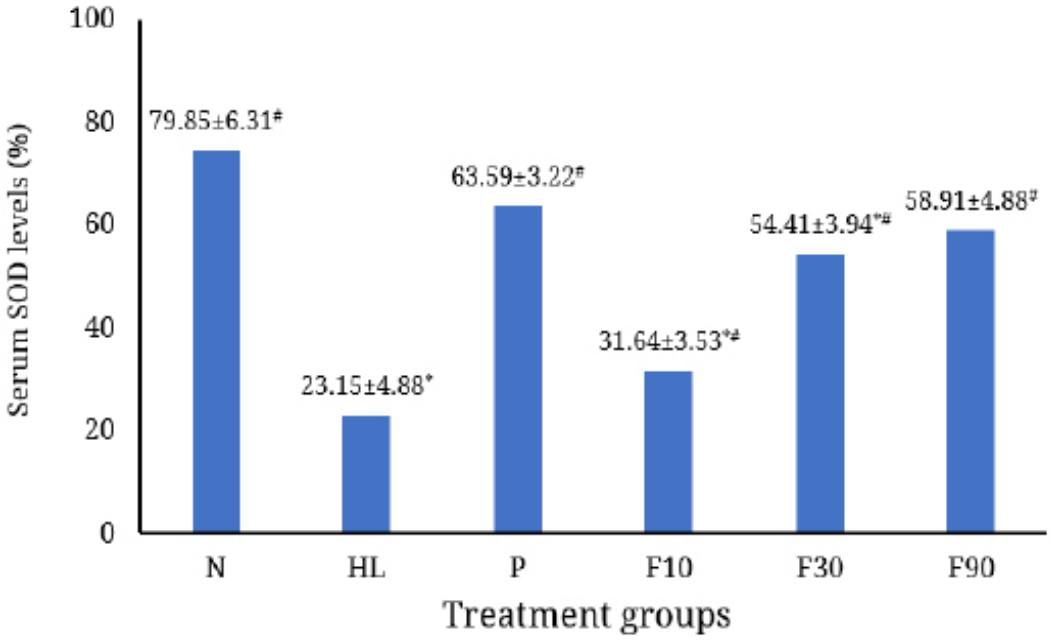
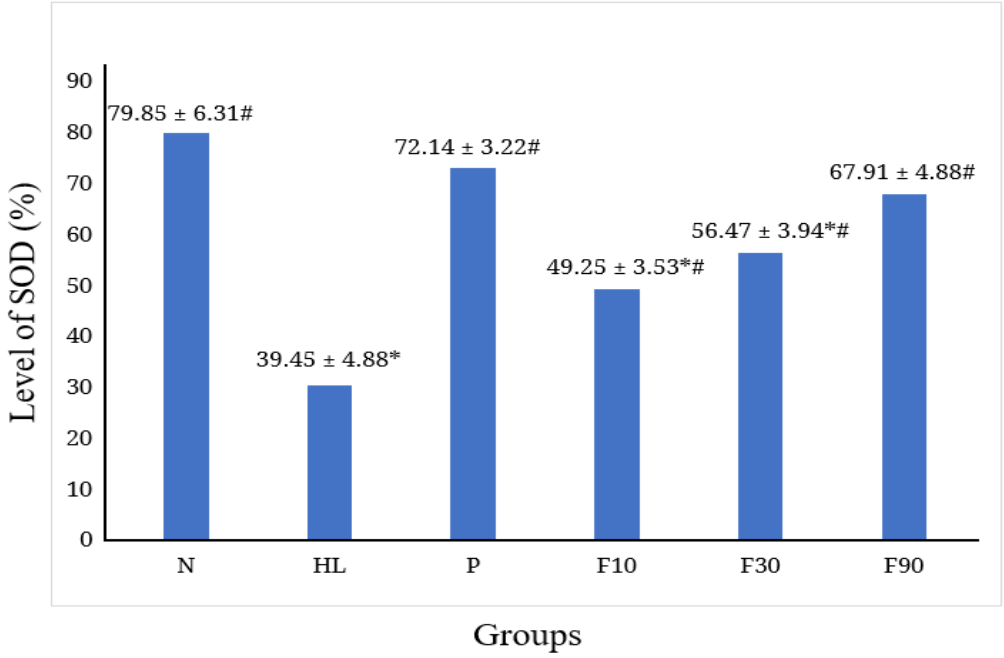


FIGURE 1. Serum SOD levels (%) in hyperlipidemic rats. N: normal, HL: hyperlipidemia, P: HL + simvastatin, F10, F30, F90: HL+ 7-OH-2-(4-OH3-methoxyphenyl)-chroman-4-one 10, 30, 90 mg/200g BW, respectively. Normality test with Shapiro-Wilk; data were tested with Anova test, Notation *: p <0.05 vs P; #: p <0.05 vs HL.

There was incorrect in Figure 1. Treatment group HL: 23.15+/- 4.88; P: 63.59+/- 3.22; F10: 31.64+/- 3.53; F30: 54.41+/- 3.94; F90: 58.91+/-4.88 it should HL: 39.45+/-4.88; P: 72.14+/- 3.22; F10: 49.25+/- 3.53; F30: 56.47+/-3.94; F90: 67.91+/- 4.88

Figure 1 correction



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There was incorrect in Figure 2

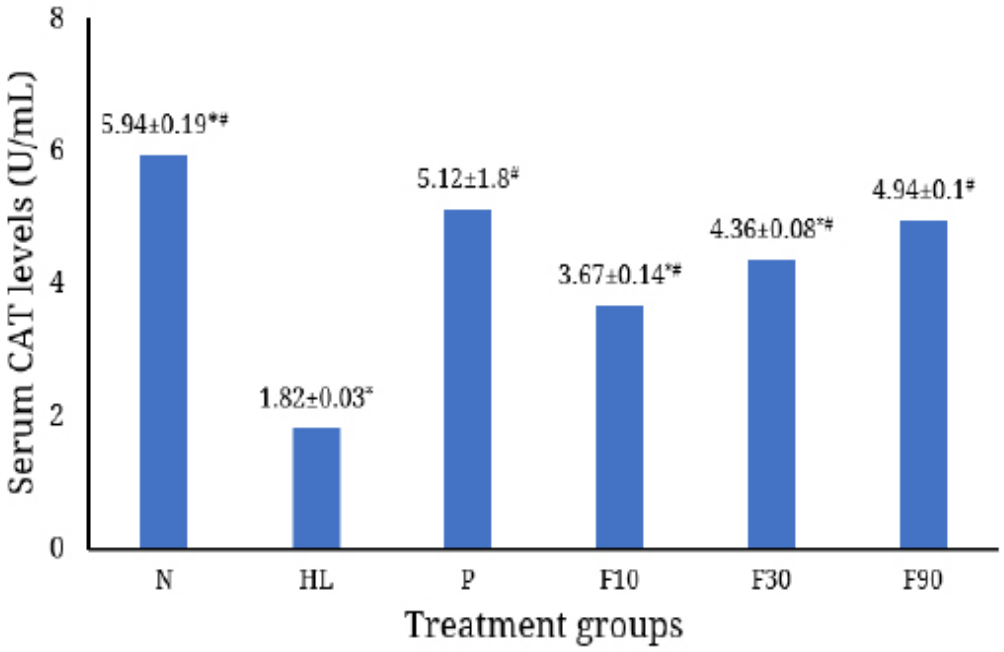

FIGURE 2. Serum CAT levels (U/mL) in hyperlipidemic rats. N: normal, HL: hyperlipidemia, P: HL + simvastatin, F10, F30, F90: HL+ 7-OH-2-(4-OH3-methoxyphenyl)-chroman-4-one 10, 30, 90 mg/200g BW, respectively. Normality test with Shapiro-Wilk; data were tested with Anova, p <0.05. Notation *: p <0.05 vs P; #: p <0.05 vs HL.

There was incorrect in Figure 2. Treatment group P: 5.12+/- 1.8 It should P: 5.12+/-0.18

Error in Figure 2

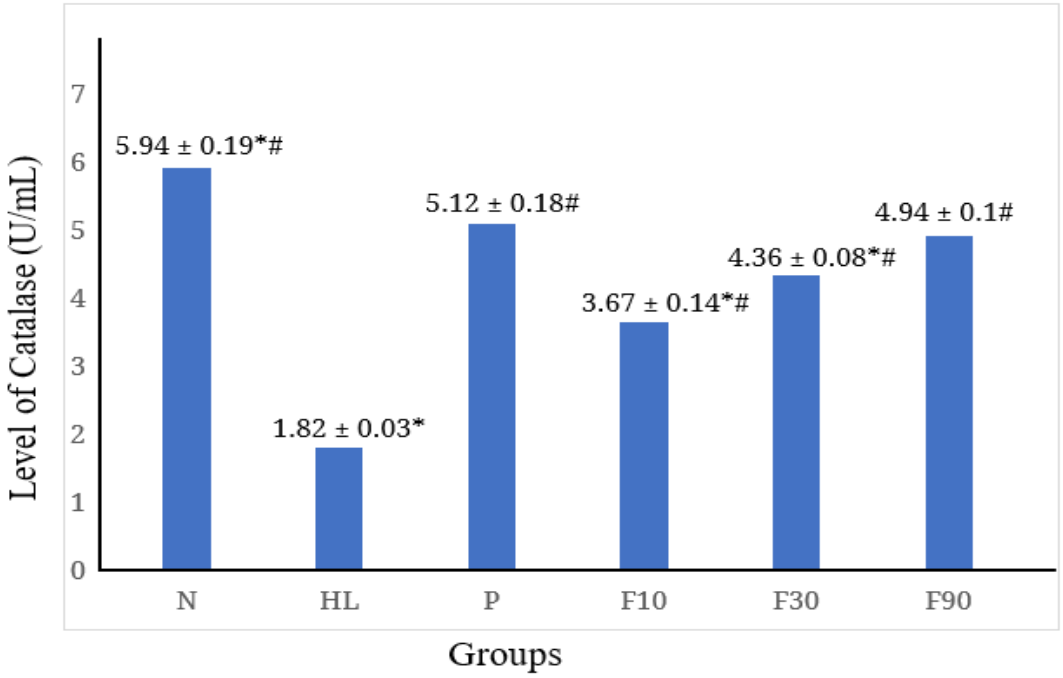

FIGURE 2. Serum CAT levels (U/mL) in hyperlipidemic rats. N: normal, HL: hyperlipidemia, P: HL + simvastatin, F10, F30, F90: HL+ 7-OH-2-(4-OH3-methoxyphenyl)-chroman-4-one 10, 30, 90 mg/200g BW, respectively. Normality test with Shapiro-Wilk; data were tested with Anova, p <0.05. Notation *: p <0.05 vs P; #: p <0.05 vs HL.

There was incorrect in Figure 3

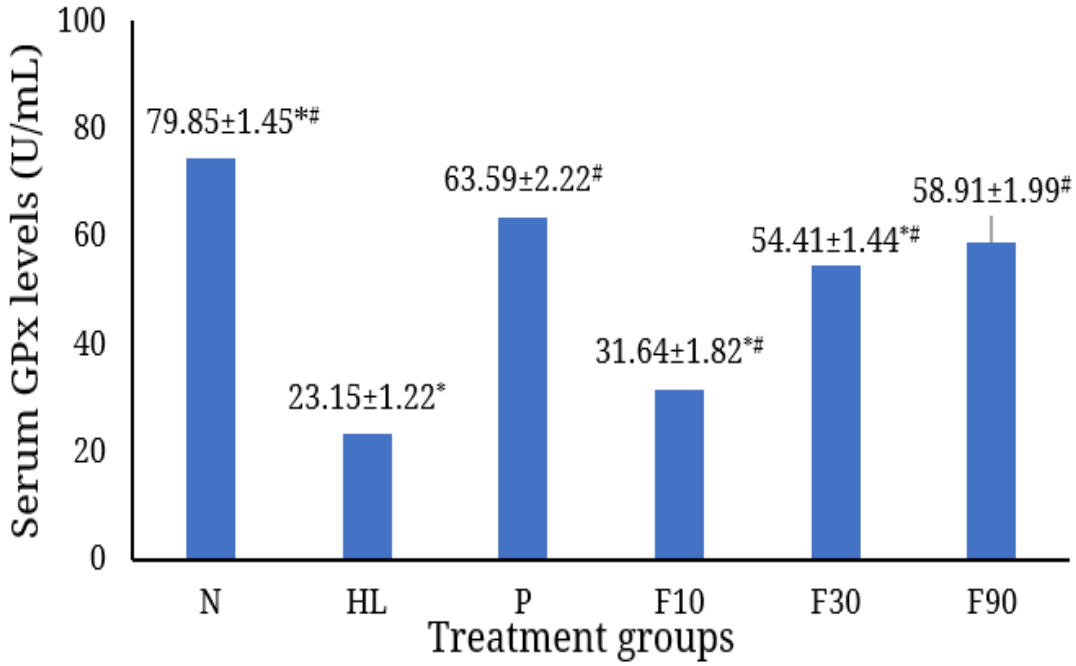

FIGURE 3. Serum levels of GPx (U/mL) in hyperlipidemic rats. N: normal, HL: hyperlipidemia, P: HL + simvastatin, F10, F30, F90: HL + 7-OH-2-(4-OH3-methoxyphenyl)-chroman-4-one 10, 30, 90 mg/200g BW, respectively. Normality test with Shapiro Wilk; data were tested with Anova p <0.05. Notation *: p <0.05 vs P; #: p <0.05 vs HL.

There was incorrect Treatment group N : 79.85+/- 1.45 It should Treatment group N : 74.47+/-1.45

Figure 3 correction

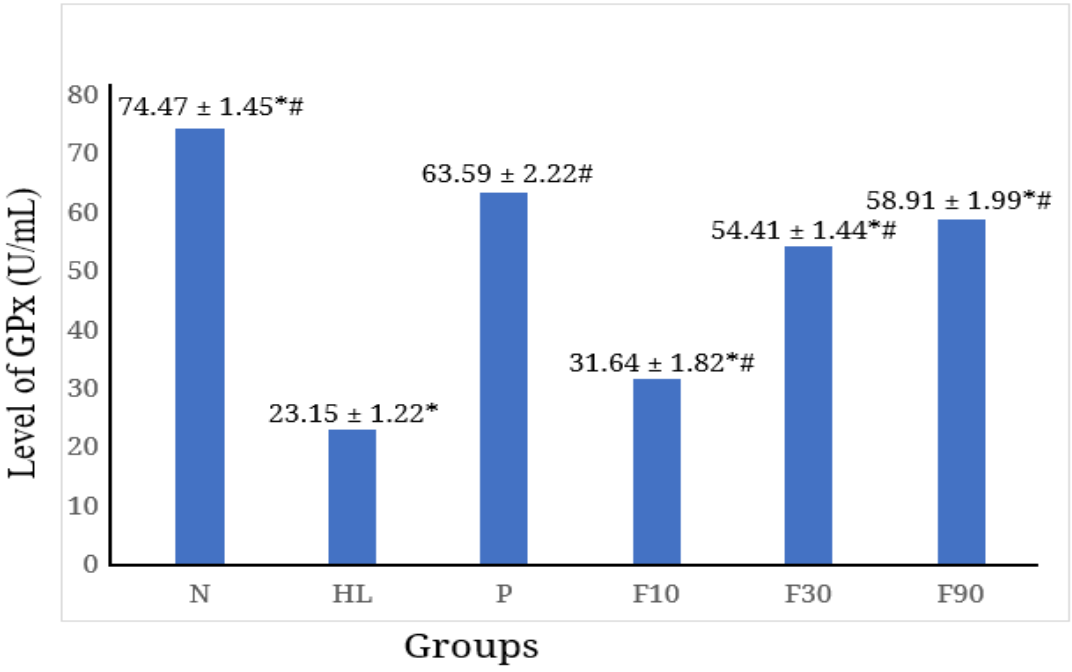

FIGURE 3. Serum levels of GPx (U/mL) in hyperlipidemic rats. N: normal, HL: hyperlipidemia, P: HL + simvastatin, F10, F30, F90: HL + 7-OH-2-(4-OH3-methoxyphenyl)-chroman-4-one 10, 30, 90 mg/200g BW, respectively. Normality test with Shapiro Wilk; data were tested with Anova p <0.05. Notation *: p <0.05 vs P; #: p <0.05 vs HL.

The authors would like to apologize for any confusion caused.