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The effect of superoxide dismutase supplementation on TNF-α and TGF-β levels in patients undergoing hemodialysis

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Abstract. – OBJECTIVE: Chronic kidney disease stage 5 on dialysis (CKD-5D) remains a global health problem associated with an increased risk of morbidity and mortality owing to cardiovascular disease. This condition is associated with chronic inflammation, which is characterized by an increase in cytokines, including tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β). Superoxide dismutase (SOD) is a first-line endogenous enzymatic antioxidant capable of neutralizing the effects of inflammation and oxidative stress. Therefore, the main aim of this study was to determine the effect of SOD supplementation on serum TNF-α and TGF-β levels in patients undergoing hemodialysis (CKD-5D).

PATIENTS AND METHODS: A quasi-experimental study with a pretest-posttest design was conducted from October to December 2021 in the Hemodialysis Unit of Dr. Hasan Sadikin Hospital, Bandung. Patients with CKD-5D who routinely underwent hemodialysis therapy twice a week were included in the study. All participants received SOD-gliadin 250 IU twice a day for 4 weeks. Serum TNF-α and TGF-β levels were assessed before and after the intervention, and statistical analyses were performed.

RESULTS: This study enrolled 28 patients undergoing hemodialysis. The median age of the patients was 42 ± 11 years, with a male-to-female ratio of 1:1. The average duration of hemodialysis in the participants was 24 (5-72) months. A statistically significant decrease in serum TNF-α and TGF-β levels from 0.109 (0.087-0.223) to 0.099 (0.083-0.149) pg/mL (p=0.036) and 15.38 ± 3.64 to 13.47 ± 3.07 pg/mL (p=0.031), respectively, after SOD administration was noted.

CONCLUSIONS: Exogenous SOD supplementation decreased serum TNF-α and TGF-β levels in patients with CKD-5D. Further randomized controlled trials are required to confirm these findings.

Key Words:
Hemodialysis, Antioxidant, Superoxide dismutase, TNF-α, TGF-β.

Introduction

Malnutrition, inflammation, and atherosclerosis syndrome (MIA syndrome) are major clinical problems in patients undergoing hemodialysis (chronic kidney disease stage 5 on dialysis [CKD-5D]) and could increase the risk of morbidity and mortality owing to cardiovascular disease (CVD). Oxidative stress, chronic inflammation, malnutrition, and subsequent endothelial dysfunction contribute to atherosclerosis[1]. Tumor necrosis factor-α (TNF-α) is a potent pro-inflammatory cytokine and an important inflammatory mediator of tissue damage[2]. Transforming growth factor-β (TGF-β) is a key mediator of renal fibrosis and plays a role in cell growth, cell differentiation and migration, formation, and degradation of extracellular matrix components. It is also a mediator of progression in CKD[3]. The increase in these cytokines is also known to play an important role in atherosclerosis[4,5]. TNF-α and TGF-b levels were elevated in patients with CKD-5D[6-8]. Chronic inflammation triggers an inflammatory chain reaction in these patients by recruiting macrophages, which then causes oxidative stress due to the accumulation of reactive oxygen species (ROS), especially superoxide radicals ($O_2^{\bullet-}$), and subsequently activates nuclear factor (NF)-κB and re-produces pro-inflammatory cytokines such as TNF-α and TGF-b[9-11]. Currently, antioxidant therapy has been used to reduce inflammation in patients with CKD-5D; however, no substantial effect is observed. Superoxide dismutase (SOD) is a first-line endogenous enzymatic antioxidant

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capable of inhibiting ROS production. SOD neutralizes inflammation by suppressing the levels of $O_2^{\bullet-}$ radicals[12,13]. No studies on the effects of SOD supplementation and its role in reducing inflammation in patients with CKD-5D were found. This study aimed to investigate the effect of SOD supplementation on TNF-α and TGFb levels in patients with CKD-5D.

Patients and Methods

The study was carried out in Dialysis Unit at Dr. Hasan Sadikin General Hospital, Bandung. We examined 28 patients on hemodialysis, who met the inclusion and exclusion criteria. Patients aged >18 years and who underwent hemodialysis (HD) twice-weekly for a minimum of 12 months were included in this study. Patients with travelling dialysis, active infection, liver diseases, acute myocardial infarction, acute congestive heart failure, malignancies, use of medications with anti-inflammatory capacity, hypersensitivity to SOD, and those receiving medications containing antioxidants were excluded from the study. During the intervention, participants who could not tolerate SOD supplementation, those who did not consume SOD supplementation for over 3 days, or those with alterations in SOD dosage were excluded. The study protocol was approved by the Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital, Bandung, and written informed consent was obtained from all patients.

The study period was set at 4 weeks, during which the patients received 250 IU of oral SOD-gliadin (twice daily after meals and under medical supervision); this supplementation was not implemented as a standard of care but as a study intervention. Patients were administered SOD-gliadin only if they agreed to participate in the study. Gliadin was administered orally to increase absorption by protecting against denaturation of SOD in the stomach. Hematological parameters were examined as baseline characteristics before the study.

Statistical Analysis

TNF-α and TGFb levels were examined before and after completion of the study period and quantified using a competitive enzyme-linked immunosorbent assay sandwich (Cloud-Clone Corp., Houston, TX, USA). Statistical analysis was performed using the paired sample *t*-test. Results are expressed as mean ± SD. Statistical significance was set at $p<0.05$.

Results

This study involved 28 patients with CKD-5D aged 42.2 ± 11.0 years who underwent dialysis for 24 (5-72) months. The mean value of HD adequacy (Kt/V) was 1.7 ± 0.3, and the etiology of CKD was dominated by hypertension (42.9%) and glomerulopathy (35.7%). The median malnutrition inflammation score (MIS) in this study was 6 (1-15) with 60.71% having an MIS >4. An MIS value >4 indicates malnutrition (Table I).

Table II shows the baseline hematological parameter of patients. TNF-α and TGF-b levels before SOD administration were 0.109 (0.087-0,223) pg/mL and 15.38 ± 3.64 pg/mL, respectively. After 4 weeks of SOD administration, the TNF-α and TGF-b levels were 0.099 (0.083-0.149) pg/mL and 13.47 ± 3.07 pg/mL, respectively. TNF-α and TGFb levels were significantly reduced in patients with CKD-5D after SOD supplementation (p=0.036 and 0.031, respectively) (Table III).

Table I. Demographics, a therapeutic regimen of the 28 hemodialysis patients.

Demographics	Patients (n = 28)
Age (years)[#]	42.2 ± 11.0
Gender (male/female)	14/14
HD duration (months)[m]	24 (5-72)
Kt/V[#]	1.7 ± 0.3
MIS	6 (1-15)
Etiology of CKD, n (%)	
Hypertension	12 (42.9)
Glomerulonephritis	10 (35.7)
Diabetes mellitus	4 (14.3)
Chronic pyelonephritis	1 (3.6)
Obstructive nephropathy	1 (3.6)
Blood Pressure[m] (mmHg)	
Systolic blood pressure	150 (110-180)
Diastolic blood pressure	90 (80-100)
Antihypertensive therapy, n (%)	
None	1 (3.6)
Single OAH	5 (17.9)
Dual OAH	19 (67.8)
Triple OAH	3 (10.7)
ESA therapy, n (%)	
Yes	20 (71.4)
No	8 (28.6)

CKD: Chronic Kidney Disease; ESA: Erythropoetin Stimulating Agents; OAH: Oral anti-hypertensive drugs. n = frequency, % = Proportion, [#]Mean ± SD, [m]Median (Min-Max).

Table II. Baseline hematological parameter of the 28 hemodialysis patients.

Hematological parameters	Value
Hemoglobin[#] (g/dL)	8.6 ± 1.8
Urea[#] (mg/dL)	163.0 ± 43.8
Creatinine[#] (mg/dL)	13.4 ± 3.4
Serum iron (Fe)[m] (µg/dL)	46.5 (23-170)
TIBC[#] (µg/dL)	185.4 ± 58.9
Transferrin saturationm (%)	28.5 (7-106)
Albuminm (g/dL)	3.57 (1.71-4.14)
Asam urat[#] (mg/dL)	8.6 ± 1.7
HDL[#] (mg/dL)	41.0 ± 11.9
LDL[#] (mg/dL)	93.4 ± 24.5
Triglycerides[m] (mg/dL)	111 (18 - 620)
SGOT[#] (U/L)	14.3 ± 5.2
SGPT[#] (U/L)	18.5 ± 7.2
RBS[m] (mg/dL)	108 (17-359)

TIBC: Total Iron Binding Capacity; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum Glutamic Pyruvic Transaminase. n = frequency, % = Proportion, [#]Mean ± SD, [m]Median (min-max).

Discussion

CKD is associated with increased inflammation and oxidative stress, which plays an important role in the development of CVD, the main cause of mortality and morbidity in patients with CKD. Several risk factors that contribute to the development of CVD in patients with CKD include dyslipidemia, oxidative stress, inflammation, and endothelial dysfunction. Patients undergoing HD experience persistent inflammation and oxidative stress. These conditions are possible reasons for atherosclerotic changes[14].

TNF-α is a key pro-inflammatory cytokine synthesized in large amounts by activated macrophages and T cells and also by stressed epithelial cells and other cells. High levels of TNF-a in patients with CKD are associated with markers of malnutrition, inflammation, and higher mortality[15,16]. TGF-β is a profibrogenic cytokine that plays a role in cell growth, cell differentiation, migration, formation, and degradation of extracellular matrix components and is a mediator of progression in CKD[3]. HD provides a solution for patients with CKD, but it also plays an important role in the inflammatory process in patients with CKD-5D. Hemodialysis causes inflammation in 30-50% of patients with CKD-5D[17]. Inflammation, mediated by pro-inflammatory cytokines such as interleukin-1beta (IL-1β), TNF-a, and TGF-β, significantly increases after HD sessions[8,18]. During the dialysis procedure, blood exposure to dialyzer membranes and dialysate triggers the activation of complement factors, platelets, polymorphonuclear white blood cells, and subsequently, ROS production, but this increase is stabilized at the end of dialysis. In addition, blood exposure to dialyzer membranes causes the entry of endotoxins from the dialysate into the blood and increases the release of TNF-a in patients with CKD-5D[11]. Antioxidant supplementation, such as SOD, may be an additional therapy to reduce these conditions and improve outcomes in patients undergoing hemodialysis. Supplementation with SOD decreased TNF-a levels in the non-renal patient groups, but there were no data on TGF-β levels.

Several studies[6-8,19] have shown an increase in TNFa and TGF-β levels in patients with CKD-5D. Alwahaibi et al[7] reported that serum TNF-a levels in patients with CKD-5D and kidney transplant were significantly higher than that of the healthy participants (40.2 ± 10.8 *vs.* 5.2 ± 1.7 pg/mL; $p<0.05$ and 16.1 ± 5.9 *vs.* 5.2 ± 1.7 pg/mL; $p<0.05$). Samy et al[19] also reported that serum TNF-a levels in diabetic and non-diabetic patients with CKD-5D were significantly higher than those in the control group (51.89 ± 10.86 *vs.* 30.94 ± 13.13 pg/mL; $p<0.001$ and 40.28 ± 8.52 *vs.* 30.94 ± 13.13 pg/mL; $p<0.001$). Furthermore, Kir et al[6] showed that TNF-a levels in patients before undergoing dialysis and patients undergoing continuous ambulatory peritoneal dialysis and HD were significantly higher than those in the control group (17.24 ± 9.22, 31.57 ± 10.56, 24.34

Table III. TNF-α and TGF-β levels before and after administration of SOD.

Variable	Before	After	*p*
TNF-α (pg/ml)	0.109 (0.087-0.223)	0.099 (0.083-0.149)	0.036*
TGF-β (pg/ml)	15.38 ± 3.64	13.47 ± 3.07	0.031**

TNF-α: tumor necrosis factor-alpha; TGF-β: Transforming growth factor-beta. *Wilcoxon test, **Paired *t*-test, Statistically significant at $p < 0.05$.

± 5.32, and 7.64 ± 4.12 pg/mL, $p<0.001$, respectively). A study by Avci et al[8] showed that TGF-β levels were significantly higher in patients with CKD-5D than in controls ($p<0.05$). Our study showed that TNF-a and TGF-β levels were higher in patients with CKD-5D before SOD administration. There were no data regarding the effects of SOD supplementation on inflammation status in patients with CKD-5D.

Table III shows a decrease in the median value of TNF-a levels from 0.109 (0.087-0.223) pg/mL to 0.099 (0.083-0.149) pg/mL ($p=0.036$) and a decrease in the mean value of TGF-β levels from 15.38 pg/mL to 13.47 pg/mL ($p=0.031$) after SOD administration. The decrease in TNF-a and TGF-β levels after SOD administration in patients with CKD-5D proved that SOD supplementation could reduce chronic inflammatory processes that are commonly found in these patients. No study has investigated the effect of SOD supplementation on reducing TNF-a and TGF-β levels in patients with CKD-5D. Studies by Vouldoukis et al[20] showed that Cantaloupe melon extract (CME) supplementation, which contains 5 IU/nitroblue tetrazolium (NBT) of SOD, significantly reduced TNF-a levels ($p<0.01$) and increased the production of the anti-inflammatory cytokine IL-10 ($p<0.01$) in mice. This indicates that SOD activity in CME regulates macrophage activation, as indicated by the decrease in the production of pro-inflammatory cytokines: TNF-a, IL-1β, and IL-6, which inhibits neutrophil infiltration and can enhance the innate immune response by increasing the production of the anti-inflammatory cytokine IL-10[20,21].

Hemodialysis adequacy (Kt/V) in our study was 1.7 ± 0.3, which is lower than the Indonesian Society of Nephrology (PERNEFRI) and international guidelines recommended values. A study by Samy et al[19] demonstrated that low Kt/V increases TNF-a levels; hence, HD adequacy is crucial. Inadequate HD causes uremia, which stimulates T cells to produce pro-inflammatory cytokines such as TNF-a and high-sensitivity C-reactive protein (hs-CRP)[6]. Several studies[22-24] have shown that inflammatory states influence the development of renal anemia. Zhai et al[22] showed that TNF-a and IL-6 levels in patients with chronic anemia were significantly higher than those in the non-anemia group ($p<0.01$). Pro-inflammatory cytokines have been shown to influence erythropoiesis at several levels, including suppression of erythroid progenitor cell proliferation[23]. Allen et al[24] demonstrated the inhibition of erythroid colony formation by soluble factors in the serum of patients with CKD and inflammatory disease. Previous studies[25,26] have also shown that TNF-a induces hypoproliferative anemia through direct effects on erythroid progenitor cells, indirect stimulation of IFN-γ production, and inhibition of growth in the later stages of erythropoiesis, which is the colony-forming unit of erythroids. Inflammatory state also influences erythropoiesis by inhibiting hypoxia-induced erythropoietin (EPO) production in Hep3B cells. In addition, clinical and experimental evidence have also shown that inflammation contributes to patients' poor response to erythropoietin-stimulating agent (ESA) therapy[27,28].

Keithi-Reddy et al[29] explored the relationship between anemia and ESA therapy with inflammatory cytokine levels in patients with CKD and found that TNF-a levels were significantly higher in anemic patients than in non-anemic patients ($p<0.05$). It was also found that anemic patients who received ESA therapy had a higher probability of the upper two quartiles of increased TNF-a, IL-6, and IL-8 levels than that of non-anemic patients. In addition to inhibiting erythropoiesis, inflammation in CKD can also reduce iron availability and hepcidin production[23,29].

The majority of the participants in this study had developed anemia [mean hemoglobin (Hb), 8.6 ± 1.8 g/dL] (Table II), with a mean serum iron level of 46.5 (23-170) µg/dL. Low Hb levels may be due to inflammation that overcomes erythropoietin resistance in these patients. This is largely owing to the influence of active inflammatory conditions that affect the development of anemia, as evidenced by the high levels of TNF-a at the beginning of the study. Additionally, as mentioned previously, inflammation contributes to patients' poor response to ESA therapy. Most participants (71.4%) in this study had received ESA therapy, but ESA administration did not improve anemia, resulting in EPO-resistant anemia.

This study revealed the benefits of using SOD as an anti-inflammatory therapy in patients with CKD-5D, low Kt/V values, and anemia. This was the first study to provide hard evidence of the benefits of SOD supplementation in patients with CKD-5D. The results of this study showed that SOD supplementation significantly reduced serum TNF-a and TGF-b levels these patients.

Limitations

The major limitation of this study was its small sample size; hence, the probability of false-posi-

tive results was increased. The nature of a non-randomized single-center trial requires further external validation before its implementation in daily practice. Several measurements might provide valuable information but were not measured in this study, including polymerase chain reaction of severe acute respiratory syndrome coronavirus 2, vitamin D, intact parathyroid hormone levels, and other inflammatory cytokines (e.g., IL-1 and IL-6).

Conclusions

Oral SOD supplementation at 250 IU twice daily for 4 weeks significantly reduced serum TNF-a and TGF-b levels. These results show that SOD administration could be used as a strategy to reduce inflammatory conditions in patients with CKD-5D.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Ethics Approval
Ethical clearance was provided by the Ethical Research Committee of Hasan Sadikin General Hospital (Number: LB.02.01/X.6.5/276/2021).

Informed Consent
All patients participating in the study had read and provided written informed consent.

Availability of Data and Materials
The data used to support the findings of this study have been included in this article.

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Authors' Contribution
Rudi Supriyadi: conceptualization, data curation, investigation, validation, supervision, writing (original draft), reviewing, and editing. Enita Rakhmawati Kurniaatmaja: conceptualization, data curation, investigation, writing (original draft), reviewing, and editing. Ian Huang: data curation, investigating, reviewing, and editing. Lilik Sukesi: data curation, reviewing, and editing. Afiatin Makmun: data curation, reviewing, and editing.

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