Role of Vitamin D and E as Antioxidants Against Cerebral Endothelial Dysfunction: An In Vivo Study in White Rats (*Rattus norvegicus*) Sepsis Model

Fauzan Fajri[1], Sukmawan Fajar[1], Denny Irmawati Hasan[1], Herlina Dimiati[2], Eka Yunita Amna[2], Nora Sovira[2], Anidar[2], Mulya Safri[2], Aris Gunawan[2]
[1]Department of Veterinary Science, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia, [2]Department of Child Health, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.
Corresponding author: arisg@mhs.usk.ac.id

ABSTRACT

Sepsis is a life-threatening condition caused by the body's response to an infection, leading to organ dysfunction. A antioxidants can help neutralize harmful free radicals that cause cellular and tissue damage through oxidative stress. Vitamin D and E are two antioxidants that have been extensively studied for their potential effectiveness. This study aims to evaluate the effectiveness of vitamins D and E in reducing oxidative stress in the cerebral vascular endothelial cells of Wistar mice in a sepsis model. The study follows an experimental design and uses a posttest with a control group. The levels of NO and SOD in 24 sepsis model mice were measured using ELISA, and the cerebral endothelial tissues were examined histopathologically. An ANOVA test was performed, followed by the Post Hoc LSD test. NO and SOD levels decreased in sepsis rats from 66.88 ± 16.59 to 88.77 ± 12.83µmol/L. Sepsis mice given vitamin D and E showed significant results on changes in NO and SOD levels ($p<0.05$). Based on the histopathological results of necrosis, inflammation, and hemorrhagic cell damage in sepsis rats reached over 50% of the field of view, significantly different from sepsis mice that had been given vitamin D and E. Sepsis mice were given vitamin D and E influenced 96.2% and 98.7% on changes in NO, SOD, and cerebral endothelial dysfunction ($p<0.05$). These findings imply that vitamins D and E may be beneficial in managing sepsis-induced cerebral endothelial dysfunction, potentially impacting the treatment and outcomes of sepsis patients.

Keywords: antioxidants, cerebral endothelium, oxidative stress, sepsis, vitamin D, vitamin E

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INTRODUCTION

Sepsis is a life-threatening organ dysfunction caused by immune dysregulation of infection (KepMenKesRI, 2021). Sepsis is associated with significant morbidity and mortality rates in children (Tan, 2019). Sepsis and septic shock are the cause of morbidity and mortality in children treated in pediatric inpatient and intensive care units. The mortality rate of sepsis in Indonesian children varies from various regions, ranging from 23.9 to 65% from 2011 to 2020. A 2015 international prevalence study that collected data from 26 countries found that the global prevalence of sepsis in pediatric intensive care units was 8.2% (Sovira et al., 2020).

The brain endothelium plays a vital role in regulating cerebral blood flow and maintaining the integrity of the blood-brain barrier. Sepsis can cause endothelial dysfunction and damage to blood vessels. Sepsis induces activation of microglial cells that cause oxidative stress on the blood-brain barrier and increases pro-inflammatory cytokines such as Tumor Necrosis Factor-α

(TNFα), interleukin (IL-1β), and IL-6. As a result, there is an elevation in vascular permeability, hypotension, and compromised blood supply to essential organs (Goodson et al., 2018). Endothelial dysfunction occurs when the layer of endothelial cells lining the walls of blood vessels is damaged, and the ability to regulate vasodilation and vasoconstriction is lost, as well as the balance between pro-inflammatory and anti-inflammatory factors. It can lead to increased vascular permeability, oedema, and hypotension, characteristics of sepsis. Endometrial dysfunction can also lead to microcirculation disorders and tissue hypoxia, which can worsen organ dysfunction and increase the risk of death in sepsis patients (Atterton et al., 2020).

The primary mediator that acts as a vasodilator substance is Endothelium Relaxing Factor (EDRF), namely Nitric Oxide (NO) or nitric oxide; in reasonable amounts, NO compounds also play an essential role in controlling the physiological process of cell communication and inflammation, but in excessive amounts, NO has the character of being reactive, genotoxic and destructive oxidative for human cells (Darwin et al., 2018).

Antioxidants play an important role in overcoming oxidative stress by neutralizing free radicals that can damage cells and tissues. SOD stands for superoxide dismutase, which is an antioxidant enzyme that plays a role in converting superoxide into hydrogen peroxide and oxygen. During sepsis, an increase in oxidative stress can lead to cell damage. SOD can help protect the body's cells from damage caused by free radicals during sepsis. During an event of sepsis, superoxide dismutase (SOD) levels in the body may increase (Nurkhasanah et al., 2023). Vitamin D can affect the immune system and regulate the inflammatory response, so it can help reduce inflammation and improve recovery in sepsis patients. Some studies suggest that vitamin D administration may improve survival and reduce the risk of complications in sepsis patients (Minter et al., 2020). Vitamin D can inhibit the production of pro-inflammatory cytokines such as TNF-α and IL-6 and increase the production of anti-inflammatory cytokines such as IL-10 (Olejarove, 2019).

Several studies show the role of Vitamin E in reducing the impact of sepsis on the body, such as reducing inflammation and cell damage caused by sepsis. For example, a study in mice found that vitamin E administration can reduce levels of pro-inflammatory cytokines and increase antioxidant levels in the blood, thereby decreasing cell and tissue damage caused by sepsis. Another human study suggests that vitamin E administration may help improve immune system function in sepsis patients (Thompson et al., 2020)

Based on this background, researchers are interested in researching the role of vitamin D and E as antioxidants against cerebral endothelial dysfunction in rat cerebral endothelial dysfunction in sepsis models. The results of this study are expected to prove that the use of vitamins D and E can improve cerebral endothelial damage in sepsis.

METHODS

Ethical approval

This research has been approved by the Medical Research Ethics Commission, Faculty of Veterinary Medicine, Syiah Kuala University, with the number Ref: 272/KEPH/X/2023. The ethical issues related to this study are the number of experimental animals, euthanasia treatment, and animal welfare.

Study period and location

The research design used was a posttest with a control group study. The research is experimental in a continuation of the previous research entitled "The Role of Vitamin C, Vitamin D, and Vitamin E in the Repair of Heart and Blood Vessel Injury: An In Vivo Study on White Rats (*Rattus norvegicus*) with a Sepsis Model." This research was conducted in the animal laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University, October 2023-April 2024.

Experimental

The calculation of the number of research samples used the Federer formula and was divided into four treatment groups, each of which amounted to 6 mice per group. The inclusion criteria included male white rats (*Rattus norvegicus*) of the Wistar strain, age 1-2 months, weight of 150-200 grams, healthy condition (active and not deformed), and rats with sepsis after LPS administration. As for the exclusion criteria, rats do not move actively, and rats die during the study. The dependent variables comprised NO, SOD levels, and endothelial dysfunction. The independent variable consisted of sepsis rats, sepsis mice with vitamin D administration, and sepsis rats with vitamin E administration.

In a phase 1 clinical study conducted at the Faculty of Veterinary Medicine laboratory at USK, experimental animals, specifically white rats, were utilized to establish a sepsis model. Sepsis was induced in the rat models using LPS, a significant component of the outer wall of gram-negative bacteria. The study involved 24 male white rats (*Rattus norvegicus*), divided into four groups: a control group, a sepsis group injected with LPS (TS), a sepsis group injected with LPS and administered vitamin D (TSD), and a sepsis group injected with LPS and given vitamin E (TSE). A group of sepsis rats was each given LPS 5 mg for five days. Then, two groups of mice were given vitamin D 7.2 IU/day and Vitamin E 5.4 IU/day for two weeks; then euthanasia was carried out on all samples. Histopathological examination was performed on brain pieces from all sample groups. Histopathological observations were made on brain cells using the CX21 Olympus electron microscope. First, take a photo of a brain preparation for treatment at five fields of view with 40x magnification of the objective lens. Second, observations were made by looking at indicators of hemorrhagic changes, necrosis, and infiltration of inflammatory cells. The last stage is to observe the photo of the preparation to assess cell damage in one field of view. The four groups of mice were also evaluated for the histopathology of cerebral endothelial cells, namely the number of necrosis and inflammatory and hemorrhagic cells. A scoring system is carried out in the histopathological assessment of the results obtained.

Data analysis

The data obtained was analyzed with SPSS version 26 (IBM Cooperation, New York, NY). The data was analyzed using ANOVA and MANOVA. The level of significance is expressed when $p<0.05$.

RESULT AND DISCUSSION

Table 1 Illustrates that rats with sepsis display higher average respiratory rates, heart rates, and body temperatures than the control group. Specifically, the values for rats with sepsis are 137.67 breaths per minute, 530 beats per minute, and 38.38°C, with standard deviations of 10.61, 21.91, and 0.48, respectively. Additionally, the sepsis rats demonstrated elevated leukocyte levels compared to the control

group. Furthermore, sepsis rats administered with vitamin D and E exhibited lower leukocyte levels than those without these supplements. Moreover, the sepsis rats experienced pleoresis and bleeding, unlike the control group and the sepsis rats receiving the supplements above.

Table 2 Shows that the highest average value of NO levels was found in control rats, 102.97 µmol/L, and the lowest in sepsis rats, 66.88 µmol/L. The highest average value of SOD was found in control rats, which was 119.02 µmol/L, and the lowest average value in sepsis rats, which was 88.77 µmol/L. Administration of vitamin D and vitamin E can increase NO and SOD levels in rats with sepsis.

Table 1. Clinical and laboratory characteristics of samples

Variable	**Mean**	**Standard Deviasi**
Breath Rate (breaths/minute)		
N	83.0	6,16
TS	137.67	10.61
TSD	136.67	13.67
TSE	140.0	8.94
Heart Rate (beats/minute)		
N	406.33	27.58
TS	530.0	21.91
TSD	538.33	21.37
TSE	536.67	21.60
Body Temperature (^{O}C)		
N	36.72	0.19
TS	38.38	0.48
TSD	38.37	0.55
TSE	38.88	0.35
Leukocyte ($10^3/mm^3$)		
N	7.83	1.36
TS	13.80	3.12
TSD	14.27	5.67
TSE	13.98	6.67
Pleoresis		
N	Negative	
TS	Positive	
TSD	Positive	
TSE	Positive	
Bleeding		
N	Negative	
TS	Positive	
TSD	Positive	
TSE	Positive	

Table 2. Distribution of NO and SOD levels in each group of mice

Variable	Mean (µmol/L)	Standard Deviasi (µmol/L)	p-value*
NO			0.02
N	102.97	13.48	
TS	66.88	16.59	
TSD	82.44	11.79	
TSE	71.87	16.61	
SOD			<0.001
N	119.02	5.09	
TS	88.77	12.83	
TSD	102.43	6.47	
TSE	91.21	10.36	

* Analysis of variance test

Table 3. Analysis of differences in NO. and SOD levels between groups in white rats (*Rattus norvegicus*) sepsis model

Variable	Group	Mean	95%CI		p-value*
			Minimum	Maximum	
NO	N vs. TS	36.08	18.29	53.87	<0.001
	N vs. TSD	20.52	2.74	38.31	0.026
	N vs. TSE	31.10	13.31	48.88	0.002
	TS vs. TSD	-15.56	-33.35	2.23	0.083
	TS vs. TSE	-4.98	-22.77	12.80	0.565
SOD	N vs. TS	30.25	19.14	41.35	<0.001
	N vs.TSD	16.58	5.48	27.69	0.005
	N vs. TSE	27.81	16.71	38.91	<0.001
	TS vs. TSD	-13.67	-24.77	-2.56	0.018
	TS vs. TSE	-2.44	-13.54	8.68	0.651

* LSD post hoc test

Table 4 Cerebral endothelial cell dysfunction

Variables	Average Scoring (%)	Standard Deviasi	p-value*
Necrosis			<0.001
N	0.33	0.00	
TS	3.33	0.33	
TSD	1.67	0.33	
TSE	1.78	0.19	
Inflammation			<0.001
N	0.22	0.19	
TS	3.67	0.33	
TSD	1.33	0.33	
TSE	1.33	0.33	
Hemorrhagic			
N	0.22	0.19	<0.001
TS	3.22	0.19	
TSD	1.55	0.39	
TSE	1.67	0.33	

* Analysis of variance test

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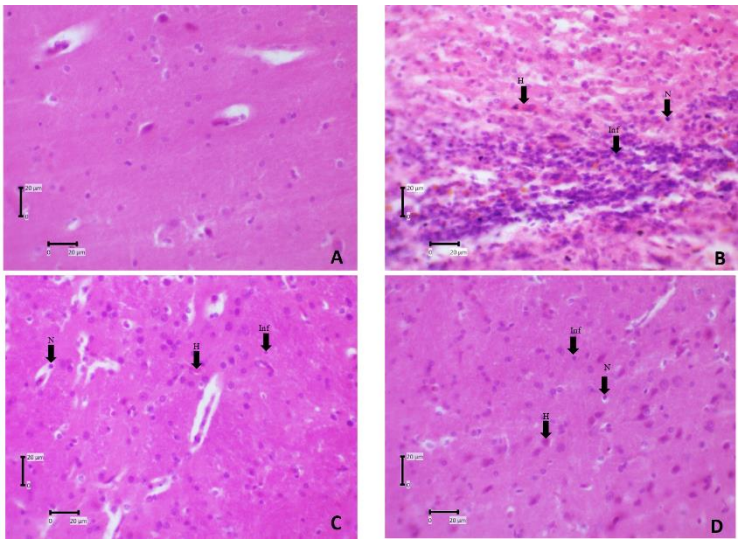

Figure 1 Histopathology of endothelial cells in the brain.
Description: Histopathological description of brain organs. Figure (A) shows the control group that is not given treatment, Figure (B) shows the group that is given LPS treatment, Figure (C) shows the treatment group that is given LPS + Vit D treatment, and Figure (D) shows the treatment group that is given LPS + Vit E treatment. All samples were subjected to HE staining and observed under 400x magnification.

Table 5. Differences in histopathological results between groups in white rats (*Rattus norvegicus*) sepsis model

Histopathology Variables	Group	Average Mean	95%CI Minimum	Maximum	p-value*
Necrosis	N vs. TS	-3.00	-3.48	-2.52	<0.001
	N vs. TSD	-1.34	-1.82	-0.86	<0.001
	N vs. TSE	-1.45	-1.93	0.97	<0.001
	TS vs. TSD	1.67	1.19	2.14	<0.001
	TS vs. TSE	1.55	1.07	2.03	<0.001
Inflammation	N vs. TS	-3.45	-4.02	-2.87	<0.001
	N vs. TSD	-1.11	-1.69	-0.54	0.002
	N vs. TSE	-1.11	-1.69	-0.54	0.002
	TS vs. TSD	2.33	1.76	2.91	<0.001
	TS vs. TSE	2.33	1.76	2.91	<0.001
Hemorrhagic	N vs. TS	-3.00	-3.54	-2.45	<0.001
	N vs. TSD	-1.33	-1.88	-0.79	<0.001
	N vs. TSE	-1.45	-1.99	-0.90	<0.001
	TS vs. TSD	1.67	1.12	2.21	<0.001
	TS vs. TSE	1.55	1.09	2.09	<0.001

* LSD post hoc test

Table 6. Administration of vitamin D to changes in NO, SOD levels and histopathological picture of cerebral endothelial cells in white rats (*Rattus norvegicus*) sepsis model

	Control (N)	**Sepsis (TS)**	**Sepsis + Vitamin D (TSD)**	**p-value***
NO level (µmol/L)	102.96 ± 13.48	66.88 ± 16.59	82.44 ± 11.79	0.02
SOD level (µmol/L)	119.02 ± 5.09	88.77 ± 12.83	102.43 ± 6.47	
Endothelial dysfunction				
Necrosis	0.33 ± 0.00	3.33 ± 0.33	1.66 ± 0.33	
Inflammatory	0.22 ± 0.19	3.66 ± 0.33	1.33 ± 0.33	
Hemorrhagic	0.22 ± 0.19	3.22 ± 0.19	1.55 ± 0.38	

*Multivariate general linear model: Wilks's Lambda, *Partial Eta Squared* = 0.962

Table 7. Administration of vitamin E to changes in NO, SOD levels and histopathological picture of cerebral endothelial cells in white rats (*Rattus norvegicus*) sepsis model

	Control (N)	**Sepsis (TS)**	**Sepsis + Vitamin E (TSE)**	**p-value***
NO level (µmol/L)	102.96 ± 13,48	66.88 ± 16.59	71,87 ± 16.61	0.002
SOD level (µmol/L)	119.02 ± 5.09	88.77 ± 12.83	91.21 ± 10.36	
Endothelial dysfunction				
Necrosis	0.33 ± 0.00	3.33 ± 0.33	1.78 ± 0.19	
Inflammatory	0.22 ± 0.19	3.66 ± 0.33	1.33 ± 0.33	
Hemorrhagic	0.22 ± 0.19	3.22 ± 0.19	1.66 ± 0.33	

*Multivariate general linear model: Wilks's Lambda, Partial Eta Squared = 0.987

The results in Table 3 show that NO levels varied significantly ($p<0.05$) between the sepsis rat group, sepsis rats given vitamin D and vitamin E, and the control group of mice. The average variances were 36.08 µmol/L, 20.52 µmol/L, and 31.10 µmol/L, respectively. There was no significant difference ($p>0.05$) between the sepsis rat group and the sepsis rat group that received either vitamin D or E, with average variances of 15.56 µmol/L and 4.98 µmol/L, respectively. The rats given both vitamins showed no significant difference, with an average NO level of 10.57 µmol/L ($p>0.05$). Additionally, the analysis revealed no significant difference in SOD levels between the treatment groups. However, the sepsis rat group and sepsis rats given vitamin D and E injections showed a significant difference in SOD levels ($p<0.05$) compared to the control group of mice, with average differences of 30.25 µmol/L, 16.58 µmol/L, and 27.81 µmol/L, respectively. Moreover, the sepsis rat group with vitamin D supplementation exhibited a significant difference ($p<0.05$) compared to the sepsis rat group and the sepsis rat group with vitamin E supplementation, with averages of 13.67 µmol/L and 11.22 µmol/L, respectively. However, there was no significant difference ($p>0.05$) between the sepsis rat group and the sepsis rat group that received vitamin E, with an average difference of 2.44 µmol/L.

Sepsis triggers a systemic inflammatory response that activates endothelial cells, leading to a cascade of pro-inflammatory signaling events. This activation increases endothelial permeability, excessive leukocyte recruitment, and microvascular thrombosis, ultimately contributing to

organ damage and dysfunction (Dolmatova et al., 2021). The inability of a vessel to dilate fully, resulting from reduced NO bioavailability, is known as endothelial dysfunction. This condition is associated with increased levels of endothelin-1 (ET-1) and decreased levels of NO (Jimenez et al., 2023). Our study found that in rats with sepsis, the average NO level was lower than in other groups. Nitric oxide (NO) levels may experience a decline, mainly due to the uncoupling of endothelial nitric oxide synthase (eNOS). This study demonstrates that NO production can diminish under specific circumstances, such as endothelial dysfunction, due to its conversion into ONOO- (peroxynitrite), leading to heightened oxidative stress and vascular dysfunction (Joffre et al, 2021). Also, giving sepsis patients vitamin D and E can increase their NO levels. Giving sepsis patients vitamin D can raise the amount of nitric oxide in their bodies, because of vitamin D helps make more enzymes that change vitamin D into an active form that affects nitric oxide production (Touskova et al. 2019). Similarly, another study shows that giving sepsis patients vitamin E can raise their nitric oxide levels. Vitamin E protects against various conditions, including high nitric oxide levels (Mehvari et al. 2016).

Our study findings showed that the average SOD level was lower in septic rats compared to other groups of rats. Superoxide dismutase (SOD) is an important enzyme that helps protect the body from oxidative stress. However, under certain conditions, such as endothelial dysfunction and oxidative stress, the levels of SOD can decrease. This decrease in SOD levels can contribute to an increase in oxidative stress, which is often observed in conditions such as sepsis and other inflammatory diseases. This can further exacerbate the negative impact of these conditions on the body. (Bertozzi et al, 2024). This finding aligns with a study demonstrating that LPS induces a notable increase in oxidative stress, characterized by reduced levels of superoxide dismutase (SOD) in the murine brain. The diminished SOD levels contribute to the accumulation of reactive oxygen species (ROS), thereby potentially causing cellular and tissue impairment. The study assessed the protective capabilities of various antioxidants, including vitamin E, beta-carotene, and N-acetyl cysteine, postulated to mitigate the oxidative damage precipitated by LPS (Kheir-Eldin et al., 2001). Sepsis leads to increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can overwhelm the antioxidant defense systems, including SOD. This imbalance results in the depletion of SOD as it is consumed in the process of neutralizing excess superoxide radicals. Additionally, the inflammatory response associated with sepsis can lead to the downregulation of antioxidant enzyme expression, further contributing to reduced SOD levels and exacerbating endothelial dysfunction. (Joffre et al, 2021). Superoxide dismutase (SOD) activity was significantly decreased in septic mice compared to control groups. This reduction in SOD activity led to an increase in oxidative damage, which was associated with organ dysfunction. The findings underscore the critical role of SOD in mitigating oxidative stress during sepsis and highlight the potential for antioxidant therapies in managing sepsis-related organ injury (Prabhu et al., 2017). vitamin D administration led to a significant increase in SOD levels, which had been decreased due to the septic condition. This increase in SOD was associated with reduced oxidative stress and improved overall outcomes in

the septic mice, suggesting that vitamin D may help restore antioxidant defenses during sepsis (Bhat et al., 2021).

The microscopic observations, as depicted in Figure 1, reveal that the brain cells in the control group appear normal. Meanwhile, the LPS treatment group in the exact figure displays a broad distribution of inflammatory cells in the brain, accompanied by hemorrhaging at multiple sites and neurons undergoing necrosis. In images D and E, inflammatory cells are significantly reduced. There are no visible areas of inflammatory cell accumulation in the brain's field of view. Although neurons undergoing necrosis are still observable, they are present in tiny numbers. Hemorrhagic lesions are still present in this treatment group but in smaller numbers within the field of view.

Table 4 indicates that the average scoring of necrosis cells in sepsis mice is highest at 3.33, suggesting that cell damage exceeds 50% of the field of view. On the other hand, the average scoring value of necrosis cells is lowest in control mice at 0.33, indicating minimal damage to cerebral endothelial cells. The highest average inflammatory cell scoring was also found in the sepsis rat group, at 3.66. Similarly, the average hemorrhagic cell scoring in the sepsis group mice was higher than the other three treatment groups at 3.22. The average results of inflammatory and necrosis cell scoring showed that cell damage reached more than 50% of the field of view in sepsis mice. In sepsis mice fed vitamin D and E, the histopathological results showed an average scoring of less than 2, meaning that the cell damage did not reach 25% of the field of view. The results of the ANOVA-F test on the histopathological picture of the treatment group had a significant difference ($p<0.05$), so H1 was accepted. Vitamin D and E administration can reduce the risk of inflammation, necrosis, and hemorrhage in septic rats.

Effects of sepsis on the blood-brain barrier (BBB) and reported significant histopathological changes in brain endothelial cells, including increased permeability and morphological alterations. This study utilized various histological techniques to assess the impact of sepsis on the integrity and function of the brain endothelium (Zhang et al., 2014). Sepsis can cause damage to various organs, including the brain, through mechanisms involving oxidative stress and inflammatory reactions. NAC, an antioxidant, is given to mice with sepsis to evaluate its ability to reduce tissue damage. The results showed that NAC could reduce cell damage and necrosis due to sepsis and reduce the survival of mice exposed to sepsis. This study highlights the importance of the role of oxidative stress in the pathogenesis of sepsis and the potential of antioxidant therapy in reducing organ damage. (Villa et al. 1995). Damage of the cerebral endothelium is associated with the disease severity and prognosis of sepsis patients (Tacone et al. 2019).

Table 5 presents the results of the analysis of differences in the picture of necrosis between treatment groups. The picture score of cerebral endothelial cells that underwent necrosis in the control mouse group had significant results over the other three groups of mice ($p<0.001$), and the highest cerebral endothelial cell damage point was seen between the control mouse group and the sepsis mouse group, which was an average difference of 3 points. The picture of cerebral endothelial cell necrosis in the sepsis rat group also showed a significant difference from the rat group that received vitamin D and vitamin E supplementation, with an average difference of 1.67 points and 1.55 points, respectively. There was no

significant difference (p>0.05) in the picture of cerebral endothelial cell necrosis between the sepsis mice group supplemented with vitamin D and E.

The image score of inflamed cerebral endothelial cells in the control mouse group had significant results over the other three groups of mice (p<0.001), and the highest cerebral endothelial cell damage point was seen between the control mouse group and the sepsis mouse group, which was an average difference of 3.45 points. The picture of cerebral endothelial cell inflammation in the sepsis rat group also significantly differed from the mice group receiving vitamin D and vitamin E injections, with an average difference of 2.33 points each. There was no significant difference (p>0.05) in the picture of cerebral endothelial inflammation between the sepsis mice group fed vitamin D and vitamin E.

The hemorrhagic cerebral endothelial cell picture score in the control rat group had significant results over the other three groups of mice (p<0.001), and the highest cerebral endothelial cell damage point was seen between the control rat group and the sepsis rat group, which was an average difference of 3 points. The hemorrhagic picture of cerebral endothelial cells in the sepsis rat group also had significant differences compared to the mice receiving vitamin D and vitamin E supplementation, with an average difference of 1.67 points and 1.55 points, respectively. There was no significant difference (p>0.05) in hemorrhagic images in cerebral endothelial cells between the group of sepsis mice receiving D and vitamin E injections.

The findings of our study indicate that sepsis rats given vitamin D and vitamin E showed lower distribution of necrotic cells, inflammatory cells, and hemorrhagic lesions compared to sepsis rats not supplemented with these vitamins. This highlights the role of antioxidants in vitamin D and E in mitigating endothelial cell damage caused by sepsis. Both vitamin D and vitamin E are essential antioxidants in the human body. Vitamin D possesses antioxidant properties that shield body cells from free radical-induced damage. Similarly, vitamin E is an antioxidant, protecting body cells from oxidative damage. Administering vitamin D to septic mice significantly increased NO levels, previously diminished due to the inflammatory response associated with sepsis. This increase in NO was linked to improved endothelial function and reduced inflammation, suggesting that vitamin D may have a protective role in sepsis by enhancing NO production and supporting vascular health. (Khan et al., 2019). vitamin D may exert protective effects by enhancing NO synthesis and modulating the inflammatory response during septic conditions (Bhan et al., 2020). Vitamin E can curtail the formation of peroxide radicals and interleukin-6 secretion in primary microglia and the brain, potentially reducing endothelial damage and the risk of hemorrhagic disease in sepsis (Goudbout et al. 2004).

The data in Table 6 illustrates the correlation between the administration of vitamin D, the changes in nitric oxide levels, and the improvement of cerebral endothelial dysfunction in a sepsis model in white rats (*Rattus norvegicus*). The Multivariate General Linear Model (MANOVA) test was used for the analysis. The statistical analysis revealed that the administration of vitamin D had a significant 96.2% impact on the alterations in nitric oxide (NO), SOD levels, and endothelial function in the sepsis model of white rats (p<0.05).

The data in Table 7 shows the relationship between administering

vitamin E and the changes in nitric oxide levels and improving cerebrovascular endothelial dysfunction in a sepsis model using white rats (*Rattus norvegicus*). The Multivariate General Linear Model (MANOVA) test was used for analysis. Statistically, it was found that vitamin E administration had a 98.7% effect on changes in nitric oxide (NO), SOD levels, and endothelial function in the white rat sepsis model ($p<0.05$).

Oxidative stress can lead to organ dysfunction and failure. Nitric oxide depletion causes arterial constriction, while reactive oxygen species (ROS) promote a prothrombotic state. These changes reduce capillary perfusion, disrupting the delivery of oxygen to tissues. Additionally, nitric oxide is essential for maintaining endothelial function. Decreased NO levels and direct damage from ROS increase endothelial permeability, leading to tissue edema and the loss of plasma proteins into the interstitium.

Research indicates that the administration of vitamin D and E has demonstrated an effect of 96.2% and 98.7%, respectively, on changes in NO, SOD levels, and endothelial function in a sepsis model using white rats (*Rattus norvegicus*). Some limitations of the study include the small sample size, which may limit its representativeness for sepsis cases, and the short duration of the therapy, which may have affected the proper assessment of the effectiveness of vitamin D and E.

CONCLUSION

The researchers conducted in vivo studies on sepsis model mice to investigate the antioxidant effects of Vitamin D and E on cerebral endothelial damage caused by sepsis. This study found that nitric oxide and superoxide dismutase levels decrease during sepsis, which leads to endothelial dysfunction. In the histopathological analysis, mice treated with vitamin D and E showed reduced necrosis, inflammation, and hemorrhagic lesions compared to untreated sepsis mice.

REFERENCES

Atterton B, Paulino MC, Povoa P, & Martin-Loeches I. 2020. Sepsis associated delirium. *Medicina*; 56.

Bhat M, Ismail A. 2015. Vitamin D treatment protects against and reverses oxidative stress induced muscle proteolysis. *Journal of Steroid Biochemistry & Molecular Biology;* 152:171-179.

Bertozzi G, Ferrara M, Fazio AD, Maiese A, Delogu G, Fazio ND, Tortorella V, Russa RL, & Fineschi V. 2024. Oxidative stress in sepsis: A focus on cardiac pathology. *International Journal of Molecular* Sciences; 5(5):1-11.

Darwin E, Elvi EF, & Elvira D. 2018. Endotel, fungsi dan disfungsi. Cetakan ke1. Andalas University press; 38-52.

Dolmatova EV, Wang K, Mandavilli R, & Griendling KK. 2021. The efeect of sepsis on endothelium and clinical implications. *Cardiovascular Research*. 117(1):60-73.

Goodson CM, Rosenblatt K, Rivera-Lara L, Nyquist P, & Hogue CW. 2018. Cerebral blood flow autoregulation in sepsis for the intensivist: Why its monitoring may be the future of individualized care. Journal Intensive Care Medicine, 33:63–73.

Godbout JP, Berg BM, Kelley KW, & Johnson RW. 2004. α-tocopherol reduces lipopolysaccharide-induced peroxide radical formation and interleukin-6 secretion in primary murine microglia and in brain. *Journal of Neuroimmunology*, 149:101–109.

Jimenez MTB, Hadad O, Kuntic M, Daiber A, & Munzel T. 2023. Noise, air, and heacy metal pollution as risk factors for endothelial dysfunction. *European Cardiology Review*, 18:e09.

Joffre, J., & Hellman, J. 2021. Oxidative stress and endothelial dysfunction in sepsis and acute inflammation. *Critical Care,* 25(1), 389-391.

Keputusan Menteri Kesehatan Republik Indonesia. Pedoman nasional pelayanan kedokteran tata laksana sepsis pada anak. 2021.

Khan M. 2019. Vitamin D protects against sepsis-induced blood-brain barrier dysfunction in mice. *Journal of Neuroinflammation*.

Kheir-Eldin AA, Motawi TK, Gad MZ, & Abd-ElGawad HM. 2021. Protective effect of vitamin E, beta-carotene and N-acetylcysteine from the brain oxidative stress induced in rats by lipopolysaccharide. International Journal of Biochemistry & Cell Biology, 33: 475–482.

Mehvari J, Motlagh FG, Najafi, Ghazvini MRA, Naeni AA, Zare M. 2016. Effects of Vitamin E on seizure frequency, electroencephalogram findings, and oxidative stress status of refractory epileptic patients. *Advanced Biomedical Research*, 36.

Minter BE, Lowes DA, Webster NR, & Galley HF. 2020. Diferential efects of Mito vitE, α-tocopherol and trolox on oxidative stress, mitochondrial function and inflammatory signalling pathways in endothelial cells cultured under conditions mimicking sepsis. *Antioxidants*,9;195-9

Nurkhasanah, Bahri MS, & Yuliani S. 2023. Analisis antioksidan secara in vivo. Sabila GA, editor. Dalam: Antioksidan dan stres oksidatif. Yogya: UAD Press. h811-82

Olejarova M, Dobisova A, Suchankova M, Tibenska E, Szaboova K, & Koutun J. 2019. Vitamin D deficiency - A potential risk factor for sepsis development, correlation with inflammatory markers, SOFA score and higher early mortality risk in sepsis. *Bratislav Medical Journal*, 120:284–290.

Prabhu A. 2017. Oxidative stress and antioxidant status in sepsis: A review. *Journal of Critical Care*, 38: 113-118.

Sovira N, Ismi J, Trisnawati Y, Lubis M, & Yusuf S. 2020. Profil penyakit kritis di Ruang Rawat Intensif Anak RSUD Dr. Zainoel Abidin Banda Aceh. *Sari Pediatri*, 22:92-7

Taccone FS, Su F, Pierrakos C, He X, James S, Dewitte O, Vincent JL, & De Backer D. 2019. Cerebral microcirculation is impaired during sepsis: an experimental study. *Critical Care*,23:87.

Tan B, Wong JJ, Sultana R, Koh JCJW, Jit M, Mok YH, & Lee JG. 2019. Global case-fatality rates in pediatric severe sepsis and septic shock: a systematic review and meta-analysis. *JAMA Pediatri*, 173(4);352–362.

Thompson MA, Zuniga K, Sousse L, Christy R, & Gurney CJ. 2022. The role of vitamin E in thermal burn injuries, infection, and sepsis: a review. *Journal of Burn Care & Research*, 1:1260–1270.

Touskova S. 2019. Vitamin D and Nitric Oxide Production: A Review. *Current Medical Chemistry*, 26(33),5985-5993.

Villa P, & Ghezzi P. 1995. Effect of N-acetyl-L-cysteine on sepsis in mice. *European Journal of Pharmacology*, 292:341–344.

Zhang Y, Chen Y, & Liu Y. 2014. Sepsis-induced blood-brain barrier dysfunction: Mechanisms and therapeutic strategies. *Frontiers in Cellular Neuroscience*.

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