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Effect of vitamin D on free radical metabolism in renal tissues of septic rats

Septik ratların böbrek dokularında serbest radikal metabolizmasına vitamin D'nin etkisi

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Abstract: Objective: Sepsis is a common cause of morbidity and mortality in the intensive care unit. Lipopolysaccharide (LPS)-induced excessive immune response is associated with multi-organ damage in sepsis. Excessive immune response causes multi-organ damage by increasing oxidative stress. Studies on the antioxidant effects of vitamin D demonstrated its protective role. In this study we aimed to investigate the effects of vitamin D on free radical metabolism in LPS injected rats.

Methods: Twenty four wistar albino rats were separated into control, sepsis, sepsis+vitamin D and vitamin D groups. Sepsis was induced with single injection of LPS *Escherichia coli* (O111-b4) 16 mg/kg. Vitamin D was given 2 mg/kg 25 (OH) single dose via gavage for 3 days. Renal function tests were analyzed in serum. Tissue catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione-S-transferase (GST) activities were analyzed, and rat renal tissues were evaluated histopathologically.

Results: SOD and GSH-Px activities were not significantly different between the groups. CAT activities were significantly decreased in all groups compared to control, this suppression was seen in the sepsis+vitamin D group versus sepsis group. GST activities were significantly decreased in sepsis and sepsis+vitamin D group compared

to control, but GST activities were significantly elevated vitamin D group compare to sepsis and sepsis+vitamin D group. Blood urea nitrogen (BUN) and creatinine levels were significantly elevated in sepsis and sepsis+vitamin D group. Inflammation, expansion in bowman capsule were detected in sepsis and sepsis+vitamin D groups.

Conclusion: Vitamin D treatment does not seem to have protective role against renal toxicity in sepsis. Nutrition with vitamin D in sepsis may have suppressive effect on antioxidant enzymes such as CAT and GST due to reduced substrate level which use hydrogen peroxide (H_2O_2) and GSH as a substrate.

Keywords: Vitamin D, Sepsis, Renal toxicity, Antioxidant enzymes

Özet: Amaç: Sepsis, yoğun bakımlarda mortalitenin ve morbiditenin en sık nedenidir. Sepsiste lipopolisakkaridin (LPS) neden olduğu aşırı immün yanıt oksidatif stres oluşturarak organ yetmezliğine neden olmaktadır. Vitamin D'nin antioksidan etkileriyle ilgili yapılan araştırmalarda vitamin D'nin koruyucu rolünün olduğu gösterilmiştir. Biz de çalışmamızda sepsiste serbest radikal metabolizması üzerine vitamin D'nin etkisini araştırmayı amaçladık.

Metod: 24 adet dişi wistar albino rat kontrol grubu, sepsis grubu, vitamin D grubu, sepsis+vitamin D grubu olmak üzere 4 gruba bölündü. Sepsis 16 mg/kg dozunda LPS *Escherichia coli* (O111.b4) intraperitoneal yolla uygulanarak

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oluşturuldu. D vitamini 3 gün boyunca 2 mg/kg 25 (OH) Vitamin D3 (ayçiçek yağında çözülerek) gavaj yolula verildi. Böbrek fonksiyon testleri rat serumlarından çalışıldı. Doku katalaz (CAT), superoksit dismutaz (SOD), glutatyon peroksidaz (GSH-Px), glutatyon-S transferaz (GST) enzimleri ölçüldü. Dokuların histopatolojik incelemesi yapıldı.

Bulgular: SOD ve GSH-Px aktivitelerinde gruplar arasında fark görülmedi. CAT aktivitelerinde en fazla sepsis+vitamin D grubunda olmak üzere tüm gruplarda kontrol grubuna göre anlamlı baskılanma görüldü. GST aktivitesinin sepsis ve sepsis+vitamin D gruplarında baskılandığı görüldü. Bununla birlikte GST aktivitesinin vitamin D grubunda sepsis ve sepsis+vitamin D grubuna göre yüksek olduğu görüldü. Kan üre nitrojeni (BUN) ve kreatinin düzeylerinde sepsis ve sepsis+vitamin D gruplarında artış olduğu gözlemlendi. Sepsis ve sepsis+vitamin D gruplarında bowman kapsülünde genişleme ve inflamasyon görüldü.

Sonuç: Sonuç olarak sepsiste vitamin D kullanmasının renal hücreleri toksik etkiden koruyucu bir etkisinin olmadığını, buna karşılık özellikle sepsis durumunda kullanıldığında hidrojen peroksit (H_2O_2) ve GSH'yı substrat olarak kullanan anti oksidan enzimler (CAT, GST gibi) üzerine baskılayıcı etkisinin olduğunu gördük.

Anahtar Kelimeler: Vitamin D, Sepsis, Renal toksisite, Antioksidan enzimler

1 Introduction

Sepsis is an inflammatory response to the infection and affects all the systems. Mortality rate is still high although development of antimicrobial therapies and intensive care unit strategies [1]. The cause of mortality resulting from sepsis is attributed to uncontrolled activation of proinflammatory and procoagulant system which culminates in activation immune system response. Excessive immune response with hypovolemia, peripheral vasodilatation, myocardial depression, increased endothelial permeability and death may occur in sepsis [2]. Therefore, immune system modulators are in treatment practices in addition to the antibiotics [3].

In sepsis models, it has been observed that oxidant system is increased, compared with antioxidant system because of increasing in reactive oxygen species (ROS)/reactive nitrogen species (RNS) and occurring redox imbalance [4]. Glutathione peroxidase activity and reduced glutathione (GSH)/oxidized glutathione (GSSG) levels decrease in sepsis and the reason of this imbalance

in redox is may be due to suppression of glutathione synthesis [5]. At the same time, it has been identified that antioxidant enzyme activities such as superoxide dismutase (SOD) and catalase (CAT) activities are decreased. The SOD/CAT rate increases in sepsis and it has been considered that the reason of this is the inhibition of CAT by ROS [6]. The SOD/CAT imbalance shown up in sepsis causes an accumulation of hydrogen peroxide (H_2O_2) in the environment and this situation plays an important role in sepsis pathophysiology so that antioxidant system come to the agenda in the treatment of sepsis [4,6]. Two major biochemical features of sepsis are the cytokine cascade, which results in increases in concentrations of several cytokines and increased free radical generation. We focused on the free radical aspect of sepsis, evaluating the prognostic potential of antioxidant enzymes such as, SOD, CAT, GSH-Px and GST which play key roles in scavenging free radicals and thus limit oxidative injury.

In addition to the effects of vitamin D in bone and calcium metabolism, there are several functions such as regulation of immune function, hormone secretion, cellular proliferation and differentiation. It has been discovered that vitamin D effects releasing of cytokines, inhibits coagulation and delays the formation of disseminated intravascular coagulation [7–9].

The mechanism of relations between vitamin D and free radicals is not clear, vitamin D has different effects on antioxidant enzymes in various diseases [10–15]. Vitamin D increased SOD, CAT, GPx in diabetic rats [10]. Metabolites of vitamin D could increase H_2O_2 by decreasing CAT activity [11]. Our objective in this study was to research vitamin D effects on antioxidant enzyme system in sepsis. Furthermore, there is no research about the effect of vitamin D on antioxidant system in sepsis.

2 Materials and Methods

This study was carried out between 01/02/2013 and 08/02/2013 at Gazi University Laboratory Animals and Experimental Research Center and Gazi University Faculty of Medicine in the Department of Medical Biochemistry Research Laboratory. The study was approved by Gazi University Animal Experiments Local Ethics Committee with the number 6–985 on 17/01/2012.

In the experiments, 24 male wistar albino rats (2 months old) with weight of 250–350 gram were used. Rats had free access to food and water, they were kept in a room with a rhythm of 12 hour light and 12 hour darkness. Rats were randomly separated into 4 groups. (Control group,

sepsis group, 25 (OH) vitamin D3 group, 25 (OH) vitamin D3+sepsis group)

1. Control group: 6 rats were fed with normal diet and water for 1 week and beginning from fourth day, 0.75 ml/day sunflower oil was administered via gavage. Saline was given intraperitoneally on the seventh day.
2. Sepsis group: After feeding normally for 3 days, 6 rats were received 16 mg/kg LPS *Escherichia coli* O111.b4 intraperitoneally on the fourth day.
3. 25(OH) Vitamin D3 group: 6 rats were fed normally for 3 days then 2 mg/kg 25 (OH)Vitamin D3 (dissolved in sunflower oil) were administered via gavage following 3 days.
4. 25 (OH) Vitamin D3+sepsis group: Before LPS injection, 25 (OH) Vitamin D3 (2 mg/kg) were given via gavage for 3 days, at the end of the third day LPS *E. Coli* (O111.b4) (16mg/kg dose) were injected intraperitoneally.

24 hours later after LPS injection, rats were sacrificed because sepsis symptoms were seen (serous nasal and ocular discharge, red tears, tachypnea, depressed activity) The blood samples were taken on the 24th hour after the end of experiment. Rectal temperature measurements were performed immediately after anesthesia with a digital thermometer. Renal tissue was removed and cleaned with cold saline (+4°C).

One piece of tissue (including glomerular and tubular layers) was separated for histopathological examination. The remaining renal tissues were stored at -80°C until date of analysis. Blood samples were centrifuged at 3700 rpm for 7 minutes. Supernatants were stored at -20°C. Before analyzing enzymes renal tissues were homogenized.

Analytical Methods: Lowry method was used for tissue total protein measurement [16]. In renal tissue SOD activity [17], GSH-Px activity [18], catalase activity [19],

glutathione-S-transferase activity [20] were studied.

Blood urea nitrogen (BUN) and creatinin levels were assayed spectrophotometrically in rat serum. (Roche Cobas Integra 800). Renal tissues were stained with hematoxylin and eosin. Histopathological changes were evaluated under a light microscope.

2.1 Statistical analysis

Statistical evaluation of the data was performed in SPSS for Windows 15.0 software. Groups of data were compared with Kruskal-Wallis one way analysis of variance, followed by Mann-Whitney U tests. P values below 0.05 were considered significant.

3 Results

Increased body temperature, tachypnea (>85 breaths per minute) and tachycardia (>400 beats per minute) were observed in rats with sepsis. Rectal temperature was shown in Table 1. In sepsis and sepsis+vitamin D group, body temperature was significantly elevated ($p < 0.05$).

Creatinin values were detected significantly higher in sepsis+vitamin D group ($p < 0.05$). BUN levels were significantly higher in sepsis and sepsis+vitamin D group compared with other groups ($p < 0.05$). The maximum increase was seen in sepsis+vitamin D group. Renal function test results were shown in Table 2.

Antioxidant enzyme activities in rat renal tissues are shown in Table 3. There was no significant difference in SOD and GSH-Px enzyme activities between the groups. CAT activity was decreased in sepsis and sepsis+vitamin D group ($p < 0.05$). Significant reduction of CAT activity was

Table 1: Rectal temperature.

	Control	Sepsis	Sepsis+vitamin D	Vitamin D
Temperature (°C) (Mean±SD)	33±0.6	35.2±0.2 ^{ad*}	35.1±0.4 ^{ad*}	32.2±0.2 ^{bc*}

* $P < 0.05$ ^acompared to control group, ^bcompared to sepsis group, ^ccompared to sepsis+Vit. D group, ^dcompared to Vitamin D group.

Table 2: Renal function test results.

	Control	Sepsis	Sepsis+vitamin D	Vitamin D
BUN (mean±SD) (mg/dl)	59.2±7.4	85.06±18.1 ^{ad*}	191.1±75.9 ^{ad*}	56.2±15.1 ^{bc*}
Creatinine (mean±SD) (mg/dl)	0.42±0.08	0.46±0.0 ^{c*}	1.3±0.7 ^{abd*}	0.43±0.08 ^{c*}

* $P < 0.05$, ^acompared to control group, ^bcompared to sepsis group, ^ccompared to sepsis +Vit. D group, ^dcompared to Vitamin D group.

Table 3: Antioxidant enzyme activities in renal tissue.

	Control	Sepsis	Sepsis+vitamin D	Vitamin D
SOD (U/mg protein)	132.0±19.1	127.0±13.8	133.0±15.5	134.0±7.0
CAT (IU/mg protein)	17350±3520	13407±1449 ^{a*}	11313±1541 ^{ad*}	13539±1282 ^{ac*}
GST (IU/mg protein)	11.6±1.3	9.7±1.0 ^{ad*}	8.7±0.8 ^{ad*}	12.0±1.2 ^{bc*}
GPX (IU/mg protein)	9.3±2.7	7.8±0.47	9.1±1.5	9.6±1.5

**P*<0.05, ^acompared to control group, ^bcompared to sepsis group, ^ccompared to sepsis +Vit D group, ^dcompared to Vitamin D group.

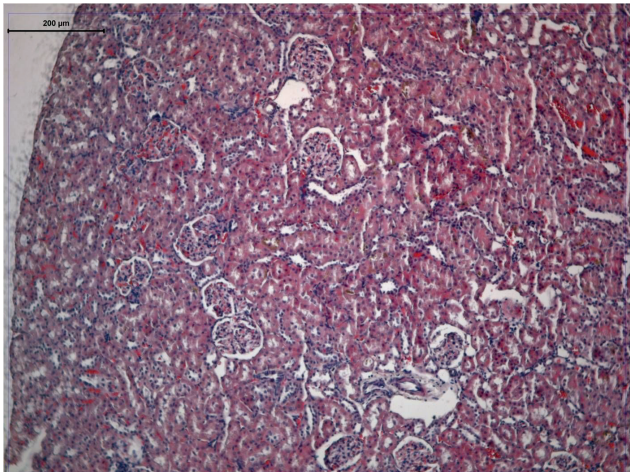


Figure 1: Control group. Renal parenchyma has a normal appearance.

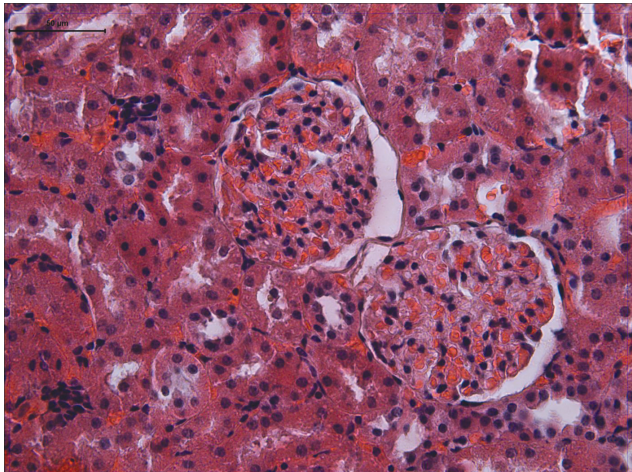


Figure 2: Vitamin D group. Renal parenchyma has a normal appearance.

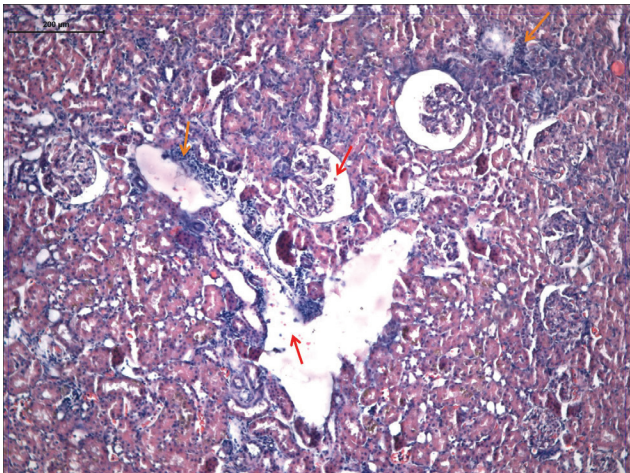


Figure 3: Sepsis group. Dilatation, retraction of glomeruli, cellular debris (showed by red arrows), and generalized inflammation of the parenchyma (showed by orange arrows).

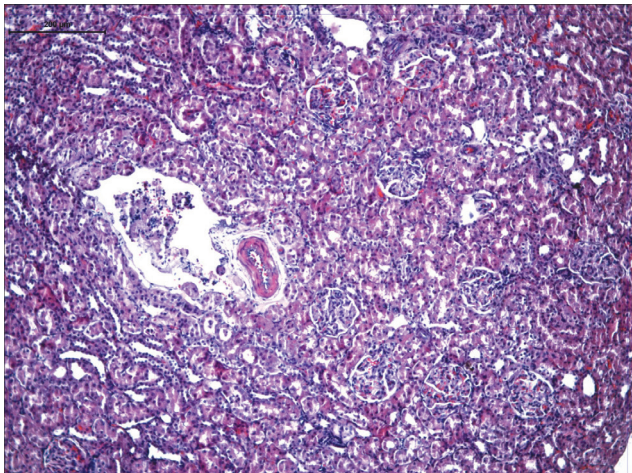


Figure 4: Sepsis +Vitamin D group. Renal parenchyma has a normal appearance.

in sepsis+vitamin D group (*p*<0.05). GST activities were significantly decreased in sepsis+vitamin D group compared to control group (*p*<0.05).The maximum decreasing activity was in sepsis+vitamin D group (Table 3).
Histopathological evaluations revealed normal appearance of renal parenchyma in control and

vitamin D group (Figure 1, 2). In sepsis group dilatation, retraction of glomeruli, cellular debris, and generalized inflammation of the parenchyma were seen extensively in bowman capsula (Figure 3). Histopathological findings in sepsis+vitamin D group indicated similar peculiarities as observed in control and vitamin D group (Figure 4).

4 Discussion

In some studies it is claimed that SOD and CAT enzymes had an imbalance, this imbalance caused excessive H_2O_2 and this was responsible for sepsis physiopathology [21]. Ritter *et al.* had mentioned SOD/CAT imbalance firstly in septic rats. They emphasized that the higher the SOD showed poor prognosis and so antioxidant therapy could be important in sepsis therapy [21]. Andrades *et al.* found an increase in SOD/CAT ratio and they showed that if this ratio got higher, sepsis become more Lethal [22]. Andrades *et al.* identified in a similar study SOD/CAT imbalance in septic rat lung and kidney tissue, they found that with antioxidant therapy SOD and CAT levels returned to basal levels. At the same time they observed no change in GPX enzyme activity in spite of sepsis [4]. Our results are compatible with the study by Andrades *et al.* In our study we observed SOD/CAT imbalance and no change in GPX enzyme activity in septic rats.

There are several studies about decrease in antioxidant enzyme activities and antioxidant therapy in sepsis [4,23]. However this is the first study about the antioxidant effects of vitamin D in sepsis.

CAT enzyme activity was suppressed seriously in sepsis, sepsis+vitamin D and vitamin D group in our study. This result is especially important for H_2O_2 metabolism. We did not find any change in SOD activity and so we thought that possible reason for increased CAT inhibition could be oxygen based radicals rather than increased H_2O_2 .

Nemere *et al.* found in their research that 24,25 (OH) vitamin D (metabolite of 1,25 (OH) vitamin D) bound CAT enzyme in intestinal cells and inactivated CAT and also increased H_2O_2 in environment [11]. In accordance with Nemere's study, serious CAT inhibition was seen in our study. We thought that 24,25 (OH) vitamin D in addition to free oxygen radicals could cause this enzyme inhibition. These results suggest that vitamin D has prooxidant effect because of CAT inhibition or has no protective effect in sepsis.

Koren *et al.* emphasized in their study on MCF-7 culture cells with breast cancer that vitamin D caused an intracellular increase of GSSG/GSH; it didn't change in GST and GSH-Px enzyme activities and therefore, due to increasing H_2O_2 vitamin D could have a prooxidant effect [12]. The explanation of this study concerning prooxidant role of vitamin D's and GSSG/GSH ratio support our results (antioxidant enzymes, kidney function tests and histopathology results) [12]. We identified inhibition of GST enzyme activity in addition to CAT enzyme activ-

ity. This situation brings to mind that these inhibitions change redox potential similar to Koren's study [12]. In another study, although vitamin D alters levels of GSSG and GSH, it had no impact on activities of glutathione dependent enzymes and this was attributed to SOD inhibition and increased H_2O_2 levels [15].

There are a lot of studies about vitamin D's antioxidant effect in several diseases, but the results are discordant with each other. Noyan *et al.* investigated antioxidant enzymes such as SOD, CAT, GPX in diabetic rats that was given 1 mg/kg/day 25 (OH) Vitamin D3 for 30 days and they found no significant differences in antioxidant enzymes in diabetes and diabetes+ vitamin D group [24]. Hamden *et al.* found an increase in antioxidant enzymes in diabetic rats that were given active form of vitamin D and so they reported that vitamin D should come into question in diabetes therapy [10]. Wang *et al.* observed decrease in SOD and CAT enzyme activities in rat heart tissue that was given calcitriol after making injury with isoproterenol [13]. Wiseman *et al.* reported that cell membrane stability was increased by vitamin D's antioxidant effect [14]. The difference in diseases, tissues and also dose of vitamin D can cause difference results between studies.

In conclusion our results are evaluated with histopathology and kidney function tests together, it is suggested that GST enzyme activity is important while researching vitamin D's protective effect in sepsis. And this result will be associated with increased GSSG/GSH ratio by vitamin D. Change in reduced/oxidized glutathione levels in environment is related with GST rather than GSH-Px. Especially this situation can be important about glutathione dependent or -SH dependent enzymes.

There are limitations in our study. We could not measure vitamin D or its metabolite levels so we don't know that dose of vitamin D is enough or not. We used renal tissue so that we don't know how vitamin D effects other tissues. Our study design was different and we didn't investigate the effect of vitamin D on mortality of sepsis.

Our study was the first study that evaluated GST and GSH-PX (using glutathione as a substrate), SOD (producing H_2O_2), CAT and GSH-Px (using H_2O_2 as a substrate) all together and supported with kidney function tests and histopathologic results. Although it is seen that using vitamin D has no protective effect about renal toxicity in sepsis, it is thought that vitamin D has a suppressive effect on antioxidant enzyme activity which use H_2O_2 and GSH metabolism.

Conflict of interest: None declared.

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