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Investigating the Reno protective effect of Vitamin D in male mice with sepsis via modulation of PI3K expression

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ABSTRACT

Objective: to explore therapeutic efficacy of vitamin D in alleviating sepsis-associated renal dysfunction through modulation of phosphoinositide 3-kinase (PI3K) gene expression. Method: 4 groups of Mus musculus mice (7 animals/ group) were assigned: Sham group: Animals have identical anesthesia and surgical procedures (laparotomy) for same duration as sepsis induction protocol but without performing cecal ligation and puncture (CLP) procedure. Control group: Mice underwent CLP procedure to induce sepsis. Vehicle group: Animals received 10% (v/v) dimethyl sulfoxide as a vehicle control. Vitamin D group: Each mouse received vitamin D (1 µg/kg) administered 30 minutes prior to CLP procedure. Results: compared to vehicle and control groups, sham group exhibited significantly lower tissue levels of TNF-α, IL-6, F2-Isoprostane, caspase-3, and KIM-1. Similarly, vitamin D group showed a marked reduction in inflammatory and oxidative stress markers. This protective effect was associated with modulation of PI3K signaling pathway, which may contribute to attenuation of renal dysfunction during CLP-induced sepsis in male mice. Histopathological analysis demonstrated that vitamin D treatment significantly ameliorated kidney tissue damage. Conclusion: Findings indicate that vitamin D therapy markedly attenuates sepsis-induced renal tissue injury in adult male mice. This protective effect appears from its pleiotropic actions, including anti-inflammatory, antioxidant, and anti-apoptotic properties. By upregulating PI3K gene expression in renal tissues, vitamin D contributes to prevention of necrosis and apoptosis, highlighting its potential as a therapeutic strategy for mitigating renal dysfunction during sepsis.

KEYWORDS: sepsis, vitamin D, TNFα, IL-6, F2 Isoprostane, caspase 3, and KIM-1, PI3K.

List of Abbreviations

PI3K	phosphoinositide 3-kinase
CLP	cecal ligation and puncture
DMSO	dimethyl sulfoxide
IL-6	Interleukin-6
TNF-α	Tumor necrosis factor
KIM-1	Kidney Injury Molecule-1
ELISA	enzyme-linked immunosorbent assay
PCR	Polymerase Chain Reaction
MIF	Macrophage migration inhibitory factor
T2DM	Type 2 Diabetes Mellitus
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
HPV	human papillomavirus
H&E	Hematoxylin-Eosin.

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INTRODUCTION

Sepsis represents a critical, life-threatening disorder that arises from an imbalanced host response to infection, resulting in systemic inflammation, immune dysregulation, and multiple organ dysfunction. Although advances in supportive medical

interventions have improved patient outcomes, sepsis continues to rank among the foremost global causes of illness and death, accounting for more than eleven million fatalities each year. The underlying mechanisms of sepsis involve intricate networks of inflammatory mediators, endothelial impairment, and immune suppression. Within these biological pathways, the phosphoinositide 3-kinase (PI3K) signaling cascade has gained prominence as a central regulator of immune and inflammatory processes during sepsis. PI3K exhibits a dual modulatory function—governing leukocyte activation, cytokine synthesis, and vascular permeability—thereby shaping the transition between the hyperinflammatory and immunosuppressive stages of the disease. Abnormalities in PI3K activity have been linked to unfavorable clinical outcomes in septic individuals, underscoring its relevance as a potential therapeutic target. Ongoing investigations are focused on fine-tuning the PI3K/Akt pathway to restore immune equilibrium and mitigate organ injury, offering promising directions for precision-based therapeutic approaches. Timely diagnosis, complemented by molecular understanding such as PI3K pathway involvement, remains essential for developing more individualized management strategies in sepsis care. Polymicrobial sepsis is a life-threatening situation characterized by multiorgan dysfunction that results from the abnormal response of the body to microbial invasion. Furthermore, it is considered the primary cause of mortality within the intensive care units [1]. Sepsis is characterized by a severe inflammatory response to infection, and its complications, including acute kidney injury, can be fatal. Animal models that correctly mimic human disease are extremely valuable because they hasten the development of clinically useful therapeutics. Too often, however, animal models do not properly mimic human disease. Discovery of antibiotics has dramatically improved the morbidity and mortality of the infectious diseases for the last decades; indeed, antibiotics and volume resuscitation are the first line of sepsis treatment strategy. However, an overwhelming inflammatory response accompanied by depression in immunological function causes multiple organ injuries and determines clinical outcomes [2]. Apart from supportive treatment (fluid management, antibiotics, vasopressors, diuretics, and dialysis), there have been a number of pharmacologic attempts directed at limiting and reversing sepsis-induced AKI. Anti-TNF therapy gained popularity particularly because of promising results in different animal models and the fact that elevated levels of soluble TNF receptors can independently predict the development of AKI and mortality [3]. The underlying pathogenesis of sepsis acute kidney injury (SAKI) is not well understood but includes oxidative stress, inflammation, and apoptosis [4]. Thus, it is necessary to rapidly decrease fundamental inflammation, which can induce serious organ damage. In the inflammatory mechanism, tumor necrosis factor-alpha (TNF-α) produced by macrophages has an important role in the infiltration of macrophages into infected sites and as a trigger for secretion of pro-inflammatory cytokines [5]. For mimicking sepsis in vivo, the cecal ligation and puncture (CLP) approach is often utilized. Because of the cecum content of bacteria, puncturing it causes polymicrobial peritonitis, then bacterial translocation into the blood (bacteremia), which if not treated can lead to septic shock, multi-organ failure, and even death [6]. Cecal ligation and puncture is widely accepted as being more realistic than other models such as injecting endotoxin (lipopolysaccharide) or even injection of pure bacteria into animals in terms of reflecting clinical reality. As a result, CLP is regarded as the golden standard for the experimental model of induction in the study of sepsis pathogenesis [7]. Attention has turned toward the role of micronutrients, particularly vitamin D, in modulating immune responses during sepsis [8]**.** Vitamin D is known not only for its role in calcium and bone metabolism but also for its immunomodulatory effects, as vitamin D receptors are expressed on macrophages, dendritic cells, and lymphocytes [9]. Low serum 25(OH)D levels have been consistently associated with higher risks of sepsis and poor outcomes in critically ill patients [8]. A recent systematic review and meta-analysis found that vitamin D deficiency increases the odds of developing sepsis by nearly twofold [10]. Furthermore, vitamin D-deficient ICU patients exhibit greater disease severity and higher mortality compared to those with sufficient levels [11]. Some interventional studies suggest that vitamin D supplementation during intensive-care stay may improve immune regulation and reduce mortality in septic patients [12]. However, results remain inconsistent due to variations in dose, timing, and baseline deficiency status. These findings highlight the need for further randomized controlled trials to clarify the therapeutic potential of vitamin D in sepsis management [9].

MATERIALS AND METHODS

This experimental study was conducted using twenty-four adult male Mus musculus mice, aged between 8 and 12 weeks, weighing 20–30 grams. The animals were obtained from the animal facility of the College of Science, University of Al-Kufa. They were maintained under controlled laboratory conditions, including a temperature of 25 ± 2°C, relative humidity of 60–65%, and a 12-hour light/dark cycle. Each group of mice was kept in separate cages with unrestricted access to standard chow and drinking water until the beginning of the experiment. All experimental procedures complied with the ethical guidelines outlined in the Guide for the Care and Use of Laboratory Animals by the Association for Laboratory Animal Science. Approval for the study was obtained from the Animal Care Committee of the University of Al-Kufa. The experimental protocols and procedures used in this study were approved in 29/8/2024 no. (20550) by ethics committee for the care and use of laboratory animals. Every effort was made to minimize animal distress, and anesthesia was induced using a combination of ketamine and xylazine during surgical interventions. After a two-week acclimatization period, the mice were randomly divided into five experimental groups:

- Sham group (negative control): animals were subjected to anesthesia and laparotomy without cecal ligation and puncture (CLP) induction.
- CLP group: mice underwent the standard CLP procedure to induce sepsis.
- Vehicle group: mice received 10% (v/v) dimethyl sulfoxide (DMSO) as a vehicle control.
- Positive control group: animals were exposed to CLP without any pharmacological treatment.
- Vitamin D (calcitriol) treated CLP group: mice were administered vitamin D (calcitriol) (1 mcg/kg) [13 14] intraperitoneally half hour prior to CLP induction [15].

An equivalent volume of vehicle was administered to the pretreated animals in the CLP group at the same time, for the same duration, and using the same method as that applied to the vehicle-pretreated group [16-17]. The cecal ligation and puncture (CLP) model was selected because it closely mimics the pathophysiological characteristics of human sepsis. This model induces polymicrobial infection derived from the animal's own intestinal flora in conjunction with necrotic tissue, thereby reflecting a clinically relevant septic condition. However, variations in surgical procedures and postoperative management may influence the

reproducibility of results across studies. Factors such as the location of ligation, the gauge of the needle, and the number of punctures can substantially affect the extent of pro-inflammatory cytokine release into the peritoneal cavity and bloodstream, ultimately altering disease severity. In this study, polymicrobial sepsis was induced using the CLP protocol. All animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg). Following shaving, a midline abdominal incision of approximately 1.5 cm was made to expose the cecum. The cecum was ligated just below the ileocecal junction and subjected to a double puncture using a 20-gauge needle [18]. Vitamin D (calcitriol) was obtained from MedChem Express (MCE, USA) and dissolved in dimethyl sulfoxide (DMSO), which served as the vehicle. The solution was further diluted with normal saline at a 1:100 ratio and freshly prepared prior to administration. A weight-based intraperitoneal dose of vitamin D (calcitriol) (1 mcg/kg) [13, 14] intraperitoneally half hour prior to CLP induction [15]. Histopathological changes in the renal cortex and the outer stripe of the outer medulla were assessed quantitatively by a blinded observer to minimize bias. The evaluated indicators of tubular injury included epithelial cell swelling, loss of the brush border, vacuolar degeneration, tubular necrosis, cast formation, and epithelial desquamation. All histological slides were examined and scored for tissue damage following the method described by Zingarelli et al. [19].

RESULTS

Effect of Vitamin D treatment on KIM after Sepsis

The resulting data showed that serum KIM had significant $p<0.05$ higher levels in sepsis and vehicles groups as compared with the sham group. While Vitamin D pretreated group showed significant $p<0.05$ lower levels of serum KIM as compared with CLP group, as shown in figure 1.

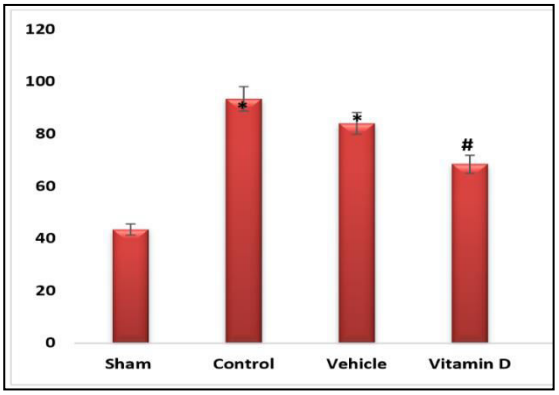

Figure 1: Mean of KIM (pg/ml) in experimental groups 24 hours after sepsis: Data are expressed as mean ± standard error; * P<0.05 versus corresponding sham; #P<0.05 versus sepsis mice

Effect of Vitamin D telmisartan treatment on TNF-alpha after Sepsis

The resulting data showed that the pro-inflammatory cytokines TNF-α had significant $p<0.05$ higher levels in sepsis and vehicles groups as compared with the sham group, while Vitamin D pretreated group showed significant $p<0.05$ lower levels of the pro-inflammatory cytokine TNF-α as compared with the CLP group as in Figure (2).

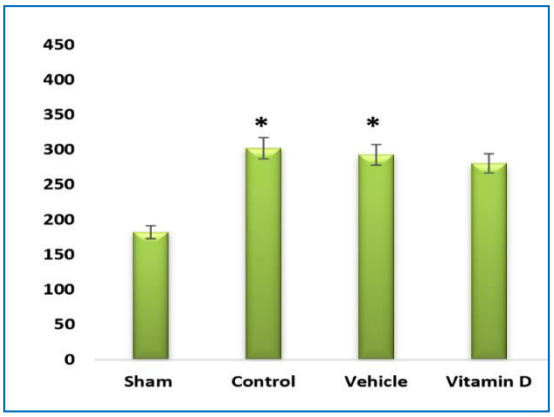

Figure 2: Mean of pro-inflammatory cytokine (ng/L) in the experimental groups 24 hours after sepsis: Data are expressed as mean ± SEM; *P<0.05 versus the corresponding sham; #P<0.05 versus sepsis mice treated

Effect of Vitamin D treatment on IL-6 after Sepsis

The resulting data showed that the pro-inflammatory cytokines IL-6 had significant $p<0.05$ higher levels in sepsis and vehicles groups as compared with the sham group, while Vitamin D pretreated group showed significant $p<0.05$ lower levels of the pro-inflammatory cytokine IL-6 as compared with the CLP group as in Figure (3).

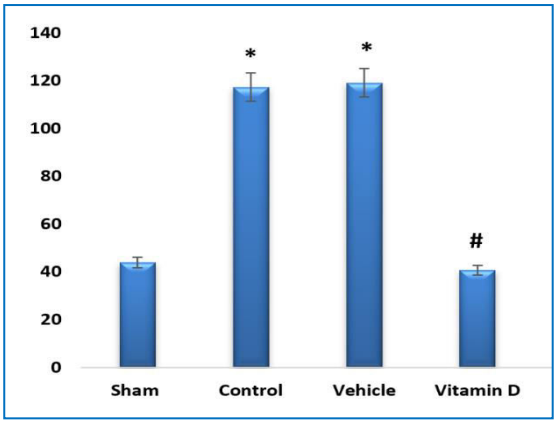

Figure 3: Mean of pro-inflammatory cytokine IL-6 (ng/L) in the experimental groups 24 hours after sepsis: Data are expressed as mean ± SEM; *P<0.05 versus the corresponding sham; #P<0.05 versus sepsis mice treated

Effect of Vitamin D on caspase-3 After Sepsis

For more documented and evident results, we carried out the tissue level of the specific injury marker caspase-3, 24 hours after polymicrobial sepsis induced by CLP model, in the experimental groups by the aid of ELISA assay protocol. ELISA outcomes demonstrated that sepsis and vehicles groups had significant p<0.05 higher tissue level when compared with sham groups, while Vitamin D pretreated groups had significant p<0.05 lower level of caspase-3 as compared with untreated sepsis group as showed in Figure (4).

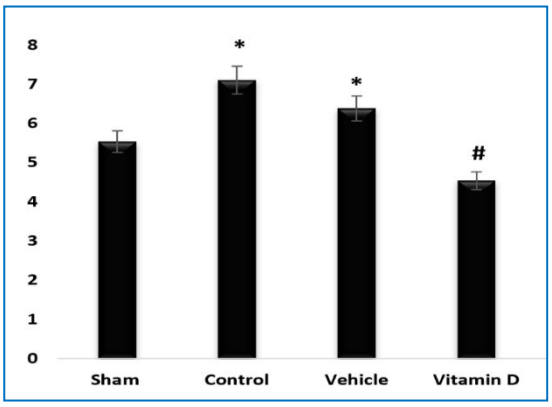

Figure 4: Mean of caspase-3 (ng/ml) in the six experimental groups 24 hours after sepsis: Data are expressed as mean ± SEM; *P<0.05 versus the corresponding sham; #P<0.05 versus sepsis mice treated

Effect of Vitamin D on MIF after Sepsis

For more documented and evident results, we carried out the tissue level of the specific injury marker caspase-3, 24 hours after polymicrobial sepsis induced by CLP model, in the experimental groups by the aid of ELISA assay protocol. ELISA outcomes demonstrated that sepsis and vehicles groups had significant p<0.05 higher tissue level when compared with sham groups, while Vitamin D pretreated groups had significant p<0.05 lower level of caspase-3 as compared with untreated sepsis group as showed in Figure (5).

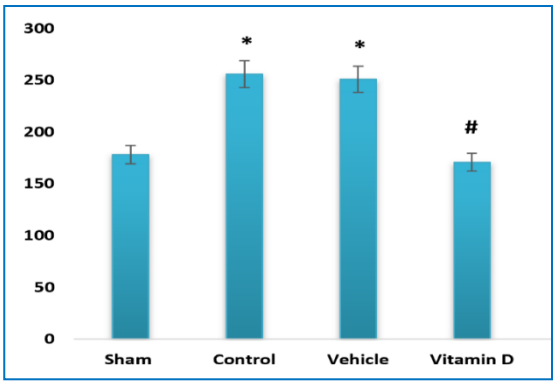

Figure 5: Mean of MIF (ng/ml) in the experimental groups 24 hours after sepsis: Data are expressed as mean ± SEM; *P<0.05 versus the corresponding sham; #P <0.05 versus sepsis mice treated

Effect of Vitamin D on F2-Isoprostan after Sepsis

For more documented and evident results, we carried out the tissue level of the specific injury marker F2-Isoprostan, 24 hours

after polymicrobial sepsis induced by CLP model, in all experimental groups by the aid of ELISA assay protocol. ELISA outcomes demonstrated that sepsis and vehicles groups had significant $p<0.05$ higher tissue level when compared with sham groups, while Vitamin D pretreated groups had significant $p<0.05$ lower level of F2-Isoprostan as compared with untreated sepsis group as showed in Figure (6).

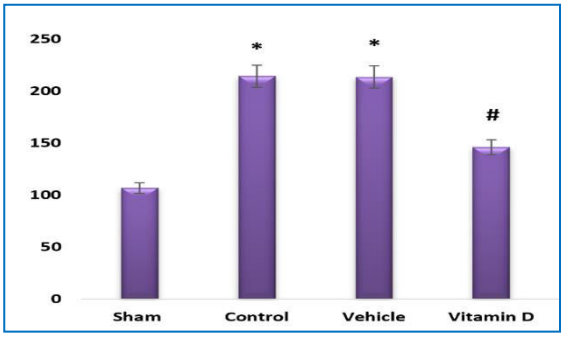

Figure 6: Mean of F-2 Isoprostane (ng/L) in the experimental groups 24 hours after sepsis: Data are expressed as mean ± SEM; *P<0.05 versus the corresponding sham; #P <0.05 versus sepsis mice treated

Effect of Vitamin D on Intracellular Signaling (PI3K) after Sepsis by PCR Technique

Furthermore; investigations on the effects of Vitamin D treatment on the intracellular signaling pathway were done. In such outcome's measurements, this study focused on modulating the effects of our treatment on PI3K signaling cascades during polymicrobial sepsis induced CLP model through the use of PCR analysis. The PI3K signaling cascades expression in renal cells had significant ($p < 0.05$) higher levels in sepsis and vehicles groups as compared with the sham group, while PI3K expression levels were significantly $p<0.05$ lower in Vitamin D pretreated groups as compared with sepsis group. PCR analysis outcomes were summarized in Figure (7).

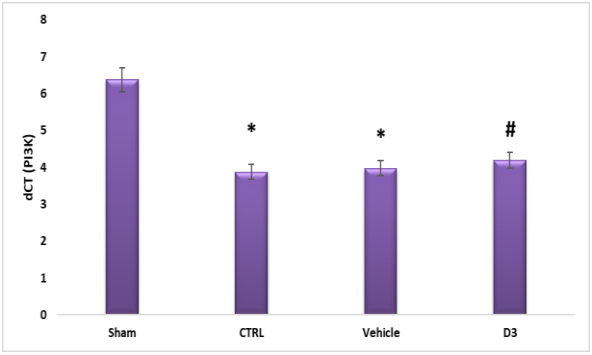

Figure 7: Mean of PI3KΔ CT in the experimental groups 24 hours after sepsis: Data are expressed as mean ± SEM; *P<0.05 versus the corresponding sham; Ψ P<0.05 versus sepsis mice; #P<0.05 versus sepsis mice treated. a lower ΔCT value indicates higher expression of the gene

Histopathological Changes of Renal Tissue following Polymicrobial Sepsis

For additional evidence on the reno-protective effect of Vitamin D histopathological study on renal tissue was done. In such histopathological study serial sections of renal tissue sections were cut after 24hours after CLP-induced polymicrobial sepsis and subjected to Hematoxylin and Eosin staining (H & E), these renal tissue sections revealed the following:

Sham Group

Sham renal tissue had nearly normal architecture without changes in erythrocyte leakage and leucocyte infiltration into renal tissue with clear tubules boundaries, all mice in this group show normal histopathological findings as shown in Figure (8).

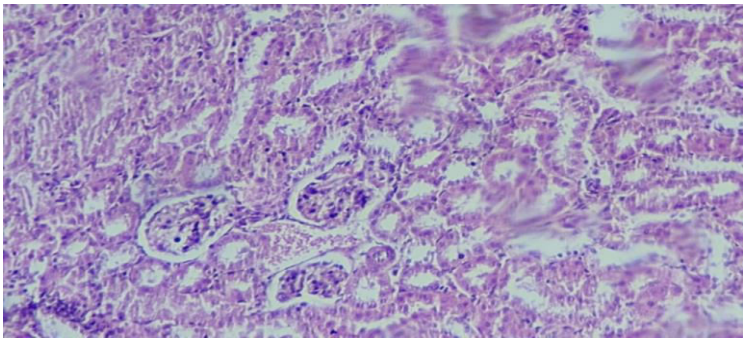

Figure 8: Histopathological section in the kidney of sham group shows normal renal tubular, the tissue is stained by H & E stain and the section is captured using light microscope and digital camera at 100X magnifier scale

Sepsis Group

Sepsis renal tissue showed a marked renal injury with the development of contraction bands and polymorphonuclear leukocytes infiltration besides interstitial edema and localized extravasation of red blood cells, these histopathological changes clearly seen in Figure (9). In terms of histopathological grading from normal renal tissue, this group showed a severe degree of damaging score.

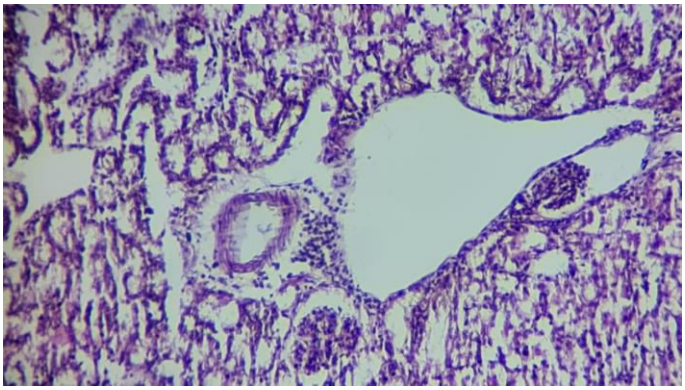

Figure 9: Histopathological sections in the kidney of the control (sepsis) group show clear renal blood vessel congestion and necrotic lesion spread in all sections. The epithelial cells of renal tubules explain an increased amount of amorphous, eosinophilic cytoplasm and small, round, dense nuclei. The tissue is stained by H & E stain and the section is captured using a light microscope and digital camera at 100 X magnifier scale

Vehicles Group

Similar to sepsis renal tissue, vehicles renal tissue exhibits renal injury with the development of contraction bands and polymorphonuclear leukocytes infiltration besides interstitial edema and localized extravasation of red blood cells, vehicle histopathological changes are well shown in Figure 10. In terms of histopathological grading from normal renal tissue, this group showed a severe degree of damaging score.

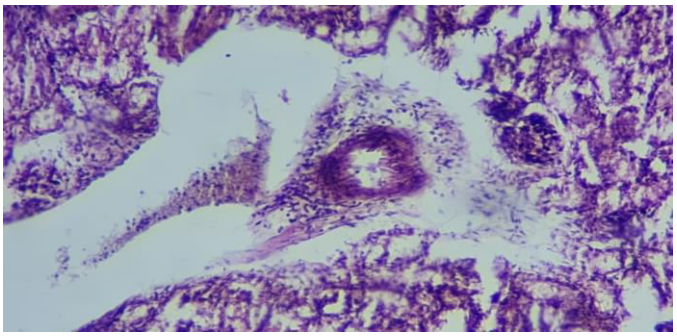

Figure 10: Histopathological section in the kidney of DMSO group shows clear renal blood vessel congestion and necrotic lesion spread in all section. The epithelial cells of renal tubules explain an increased amount of amorphous, eosinophilic cytoplasm and small, round, dense nuclei. The tissue is stained by H & E stain and the section is captured using light microscope and digital camera at 100X magnifier scale

Vitamin D Group

The histological features of the Vitamin D pretreated mice were slightly different from the sham group in which moderate architectural alterations were observed, figure (11) shows these findings. In terms of histopathological grading from normal renal tissue, this group showed mild a degree of damage

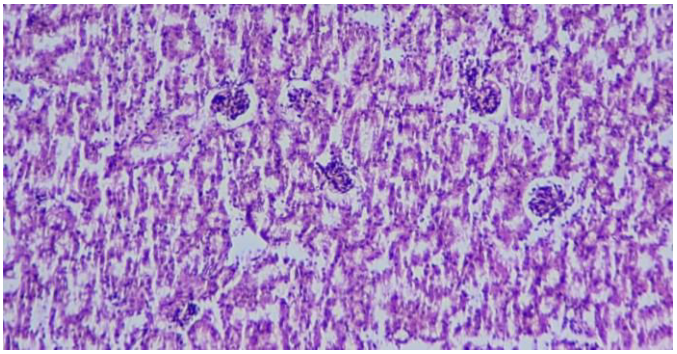

Figure 11: Histopathological section in the kidney of Vitamin D treated sepsis mice (1 mcg/Kg) shows normal glomeruli texture and mild renal tubules hypertrophy. The tissue is stained by H & E stain and the section is captured using a light microscope and digital camera at 100X magnifier scale

DISCUSSION

Sepsis is an upregulated systemic inflammatory reaction that has been identified as a global health concern [20]. One of the major complications of sepsis—a condition marked by an intense inflammatory reaction to infection—is acute kidney injury. To advance effective clinical therapies, it is essential to develop animal models that closely mimic the human manifestation of the disease. Yet, existing models frequently fail to replicate the complexity of human sepsis. The initial management of sepsis typically includes pharmacological treatment and fluid resuscitation. Over the past decades, the advent of antibiotics has markedly decreased the rates of illness and death associated with infectious diseases. Despite these advances, an exaggerated inflammatory response combined with impaired immune function often leads to damage across multiple organs, significantly influencing patient outcomes [21]. Recent evidence suggests that vitamin D deficiency is associated with an increased risk of developing sepsis, particularly among critically ill patients [22]. Individuals with low serum 25-hydroxyvitamin D levels have a significantly higher likelihood of sepsis compared with those with sufficient levels [23]. Vitamin D functions as an immunomodulator, enhancing the production of antimicrobial peptides and regulating excessive inflammatory responses, which may help reduce sepsis severity and complications [24]. However, while these associations are promising, current evidence is not yet conclusive that vitamin D supplementation alone improves sepsis outcomes; further large-scale randomized clinical trials are needed [25].

Effect of Sepsis, and Vitamin D on Kidney Injury Molecule-1 (KIM1)

Effect of Sepsis on KIM1

In this study the serum level of KIM1was significantly increased $P<0.05$ in the sepsis group as compared with the sham group. KIM1 levels increased as a result of sepsis effect of renal injury. Tu et al., 2014 in their research showed that KIM-1 are clinically useful as early biomarkers for the diagnosis of septic AKI. In addition, persistent elevation of urinary KIM-1 level may be associated with poor prognosis. As AKI remains a complex disease, the utility of combination with netrin-1, KIM-1, and other biomarkers reflecting different biologic pathways in the early diagnosis [25].

Effect of Vitamin D on KIM1

Comparing to the levels in the vehicle and control groups, this animal work proved that pretreatment with vitamin D prior to sepsis induction considerably $P<0.05$ suppresses the level of KIM1 in renal tissues. This result indicates that vitamin D has a protective impact on renal tissues and function parameters. This outcome is in alignment with other works. Few studies explained the effect of vitamin D on KIM1. One recent experimental research showed that the pretreatment with vitamin D in rats undergone renal ischemia reperfusion can attenuate the urine level of [26]. Abdul Hameed showed that calcitriol had significantly decreased levels of KIM-1, TNFα, IL-1β, F2 Isoprostane, and BAX after renal ischemia reperfusion injury [27].

Effect of Sepsis, and Vitamin D on TNF-α

Effect of Sepsis on TNF-α

In this study the serum level of TNF-α was significantly increased ($P<0.05$) in the sepsis group as compared with the sham group. TNF-α levels increased as a result of sepsis effect of renal injury. Yousif et al., 2018 exhibited that TNF-α is elevated during 24hours after sepsis induction in mice [28]. Also, Lee et al., 2021 showed that Down-regulation of TNF-α for the treatment of acute inflammatory sepsis [29].

Effect of Vitamin D on TNF-α

Comparing to the levels in the vehicle and control groups, this animal work proved that pretreatment with vitamin D prior to sepsis induction considerably ($P < 0.05$) suppresses the level of TNF-α in renal tissues. Vitamin D treated mice exhibit greater reduction in the levels of proinflammatory cytokines (IL-1β, IL-6 and TNF-α) in both myocardium tissue and plasma compared to control group (myocardial ischemia reperfusion injury by ligating the anterior left descending in heart) [15]. Abdul Hameed showed that calcitriol had significantly decreased levels of KIM-1, TNFα, IL-1β, F2 Isoprostane, and BAX after renal ischemia reperfusion injury [27].

Effect of Sepsis, and Vitamin D on IL-6

Effect of Sepsis on IL-6

In this study the serum level of IL-6 was significantly increased ($P<0.05$) in the sepsis group as compared with the sham group. IL-6 levels increased as a result of sepsis effect of renal injury. Yousif et al., 2018 showed that IL-6 is elevated during 24hours after sepsis induction in mice [28]. Wang et al., 2001 tested the role of IL-6 in sepsis-induced mucosal dysfunction in IL-6 knockout and corresponding wild-type mice; Because IL-6 deficiency may result in altered levels of other cytokines [30].

Effect of Vitamin D on IL-6

Comparing to the levels in the vehicle and control groups, this animal work proved that pretreatment with vitamin D prior to sepsis induction considerably $P<0.05$ suppresses the level of IL-6 in renal tissues. Vitamin D treated mice exhibit greater reduction in the levels of proinflammatory cytokines (IL-1β, IL-6 and TNF-α) in both myocardium tissue and plasma compared to control group (myocardial ischemia reperfusion injury by ligating the anterior left descending in heart) [15].

Effect of Sepsis, and Vitamin D on Caspase-3

Effect of Sepsis on Caspase-3

In this study the serum level of caspase was significantly increased P<0.05 in the sepsis group as compared with the sham group. Caspase levels increased as a result of sepsis effect of renal injury. Caspase-3 levels in kidney tissue are significantly (P < 0.05) greater in the CLP group compared to the sham group. These findings supported earlier research by Comim who found a large increase in the quantity of caspase-3 in apoptotic cells in septic animals as a result of caspase-3's participation in the induction of the apoptosis process [31].

Effect of Vitamin D on Caspase-3

Comparing to the levels in the vehicle and control groups, this animal work proved that pretreatment with vitamin D prior to sepsis induction considerably (P < 0.05) suppresses the level caspase-3 in renal tissues. Vitamin D maintains mitochondrial function, attenuates inflammatory response, and prevents from caspase activation [32], the antiapoptotic role of vitamin D was demonstrated in the experimental models of ischemic–reperfusion injury through reduction in the expression of caspase [33].

Effect of Sepsis, and Vitamin D on MIF

Effect of Sepsis on MIF

In this study the serum level of MIF was significantly increased P<0.05 in the sepsis group as compared with the sham group. MIF levels increased as a result of sepsis effect of renal injury. Toldi et al. research (Macrophage migration inhibitory factor as a diagnostic and predictive biomarker in sepsis: meta-analysis of clinical trials) showed MIF levels are higher with sepsis and septic shock exhibited significantly higher median plasma concentrations of MIF than did healthy controls. These results suggest that MIF can be a valuable diagnostic and prognostic biomarker in sepsis given that well designed [34], this is meta-analysis show that blood MIF levels could have diagnostic ability to differentiate between infectious and noninfectious systemic inflammation and could have prognostic value for the outcome of sepsis.

Effect of Vitamin D on MIF

Comparing to the levels in the vehicle and control groups, this animal work proved that pretreatment with vitamin D prior to sepsis induction considerably P<0.05 suppresses the level MIF in renal tissues. Pro-inflammatory cytokines as MIF were decreased 1α, 25(OH) 2D3-pretreated A549 cells by 12 h post-infection with Burkholderia pseudomallei infection [35].

Effect of Sepsis, and Vitamin D on F2-Isoprostan

Effect of Sepsis on F2-Isoprostan

In this study the serum level of F2-Isoprostan was significantly increased (P<0.05) in the sepsis group as compared with the sham group. F2-Isoprostan levels increased as a result of sepsis effect of renal injury. F2-isoprostanes are considered a key biomarker for oxidative stress and lipid peroxidation due to their stability and high specificity [36].

Effect of Vitamin D on F2-Isoprostane

Comparing to the levels in the vehicle and control groups, this animal work proved that pretreatment with vitamin D prior to sepsis induction considerably P<0.05 suppresses the level F2-Isoprostan in renal tissues. Recent randomized clinical trial conducted that the using of Calcitriol to T2DM patients can increase glutathione level; reduce MCP-1 and IL-8, lowering oxidative stress, F2-Isoprostane production and inflammation markers in those patients [37]. Abdul Hameed showed that calcitriol had significantly decreased levels of KIM-1, TNFα, IL-1β, F2 Isoprostane, and BAX after renal ischemia reperfusion injury [27].

Effect of Sepsis, and Vitamin D on the expression of PI3K

Effect of Sepsis on the expression of PI3K

PI3K modulates different signals to prevent apoptosis and promote cellular survival and proliferation in a wide variety of cell types. It has been shown that PI3K is amplified and activated in HPV-induced cervical cancers and other cancers [38]. In addition to investigation by ELISA and histopathological assessment, PI3k pathway were tested via RT-qPCR technique since this pathway involved in sepsis pathogenesis. The current study has demonstrated that the expression of PI3Kis significantly down regulated in sepsis and vehicles groups when compared with sham group.

Effect of Vitamin D on the expression of PI3K

Comparing to the levels in the vehicle and control groups, this animal work proved that pretreatment with Vitamin D prior to sepsis induction considerably P<0.05 suppresses the level PI3K in renal tissues. Vitamin D, particularly its active form 1, 25-dihydroxyvitamin D (or analogues such as paricalcitol), has been shown to modulate the PI3K/Akt signaling pathway in kidney tissue under various pathological conditions. In renal ischemia/reperfusion injury, pretreatment with paricalcitol led to activation of PI3K/Akt in kidney tissue, which was associated with reduced apoptosis, oxidative stress, and inflammation [39].

CONCLUSION

Findings indicate that vitamin D therapy markedly attenuates sepsis-induced renal tissue injury in adult male mice. This protective effect appears to result from its pleiotropic actions, including anti-inflammatory, antioxidant, and anti-apoptotic properties. By upregulating PI3K gene expression in renal tissues, vitamin D contributes to prevention of necrosis and apoptosis, highlighting its potential as a therapeutic strategy for mitigating renal dysfunction during sepsis.

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