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Endocan, a new marker for inflammation and endothelial dysfunction, increases in acute kidney injury

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ABSTRACT

OBJECTIVE: In this study, the clinical relevance of the levels of serum endocan and 25-hydroxyvitamin D [25(OH)D] were investigated in patients with acute kidney injury (AKI).The endocan or endothelial cell-specific molecule 1 (ESM-1) is a soluble proteoglycan secreted by vascular endothelial cells that plays a significant part in immunity, inflammation, and endothelial function.

METHODS: A total of 39 AKI patients (19 female, 20 male) and 38 healthy individuals (18 female, 20 male) were included in the study. The levels of serum endocan, vitamin D and other biochemical parameters were compared between the two groups.

RESULTS: In the AKI group, the values of serum creatinine, endocan, parathormone, phosphorus and uric acid were found to be higher and the total protein, albumin and calcium levels were lower compared to the control group. There was no difference between the two groups in terms of the serum vitamin D, magnesium, alkaline phosphatase and gamma-glutamyl transferase.

CONCLUSION: In patients with AKI, increased endocan is a significant marker of inflammation and endothelial injury. In addition, these patients experience vitamin D deficiency.

Keywords: Acute kidney injury; endocan or endothelial cell-specific molecule 1 (ESM 1); Vitamin D.

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Acute kidney injury (AKI) is defined as the rapid loss of renal function, which leads to the accumulation of the products of nitrogen metabolism (urea and creatinine) or a reduced urine output [1]. AKI is a syndrome that is frequently seen in hospitalized patients and has distressing outcomes. The current diagnostic method for AKI is the increased level of serum creatinine; however, due to the insensitivity of this marker to the changes in renal function, there is a need to investigate the efficiency of alternative biomarkers with a higher sensitivity and specificity to achieve an early and accurate diagnosis [2].

The endocan-specific (also known as the endothelial cell-specific) molecule 1 (ESM-1) is a soluble proteoglycansecreted by the cells of the vascular endothelium [3]. It has a significant role in inflammation, rearrangement of endothelial cytoskeleton, functioning of lymphocytes, and upregulation of several adhesion molecules [4]. Recent studies have shown that endocan can be used as a biomarker for the assessment of inflammatory, cardiovascular and chronic kidney diseases as well as cancer [3, 5–7] It is also known that inflammation and endothelial injury play a significant role in AKI pathogenesis. The impairement of the endothelial and smooth muscle cells play critical roles in the pathophysiology of AKI. When the endothelium is injured, tissue level of endocan increases [8].

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Vitamin D deficiency is commonly seen in all ages throughout the world [9] and presents as a risk factor for not only the development of infectious, autoimmune, and cardiovascular diseases, but also osteoporosis, diabetes, and cancer [10]. In chronic kidney disease, 25-hydroxyvitamin D [25(OH)D] deficiency is very common and associated with the causes of mortality and morbidity [11]. In addition, in recent years, AKI has been associated with a high risk for the progression of chronic kidney disease and end-stage renal disease [12]. It has also been reported that inflammation has a role in both vitamin D deficiency [13] and AKI etiopathogenesis [14, 15]. However, to date, only a limited number of studies have investigated the level of vitamin D in AKI.

Given the above-mentioned relationships of vitamin D 25-hydroxyvitamin D and endocan with inflammation and endothelial function, we considered these two factors to be two potential parameters that could be used as new markers for diagnosing AKI and elucidating its etiopathogenesis.

MATERIALS AND METHODS

Thirty-nine patients (19 female, 20 male) that were diagnosed with AKI at the polyclinics, emergency service and intensive care unit of our hospital between December 2015 and June 2016 and 38 (18 female, 20 male) healthy volunteers were included in the this prospective study. All the participants were above 18 years old. The exclusion criteria were having acute diseases such as myocardial infarction, sepsis, acute respiratory distress syndrome, or chronic diseases such as diabetes mellitus, rheumatic, and cardiovascular diseases and using any kind of drugs. The study protocol was performed according to the principles of the Declaration of Helsinki. All subjects gave written informed consent for participation in the study, which was approved by the clinical research local ethics committee of Erzincan University.

The AKI diagnosis was made based on the presence of one of the following; an increase in serum creatinine by ≥0.3 mg/dl within 48 hours; an increase by ≥1.5-fold from a known or assumed baseline, or a reduction in urinary output by less than 0.5 ml/kg/h over 6 hours [1].

The blood samples taken from the participants, only for patient admission at the time of enrollement in the patients with AKI, were immediately centrifuged and stored at -80 °C until use. The endocan level of each sample was measured using the ELISA method [Sun Red Human (ECSM1) ELISA kit]. The results were recorded in ng/L. The intra-assay and interassay coefficients of variation (CV) of the endocan werefound to be 10% and 12%, respectively, and the minimum detectable concentration was 7.506 ng/L.

The biochemical tests were analyzed on Beckman Coulter Olympus AU2700 analyzer, and the vitamin D 25-hydroxyvitamin D and parathormone (PTH) were measured using the Siemens Centaur XP immunoassay system. The corrected calcium was calculated with the following equation: Corrected Calcium= Serum calcium + [0.8*(4-serum albumin concentration)] [16].

Statistical analysis

The Statistical Package for Social Sciences Software for Windows version 18.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis of the demographic and clinical data of the sample. Descriptive statistics (mean, median, standard deviation, minimum, maximum, number) were generated for patients and healthy subjects. The normality assumption was checked using the Kolmogorov-Smirnov test. Differences between the two groups were evaluated by the independent t-test when the assumptions of this parametric test were met. The p-value of <0.05 was considered as statistically significant.

RESULTS

No significant difference was found between the study and control groups in terms of gender (p=0.912), weight (71±10.6 kg, 72.4±11.3 kg, p=0.589), and height (167.9±9 cm, 165.3±5 cm p=0.172) and body mass index (BMI, 25.5±2.3 kg/m^2, 27.6±4.5 kg/m^2 p=0.112); however, the mean age was significantly higher in the study group compared to the healthy individuals (72 years and 38.5 years, respectively; p<0.001). There isn't any relationship between BMI, age and levels of 25-hydroxyvitamin D, endocan (p>0.05).

In the study group, serum creatinine, BUN, endocan, PTH, phosphorus and uric acid values were found to be higher while total protein, calcium, albumin, and calcium corrected for albumin were found to be lower compared to the control group. However, there were no significant differences between the two groups in terms of the serum vitamin D, magnesium, alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) values (Table 1). According to a receiver operating characteristic (ROC) analysis based on the cut-off value of >220,

TABLE 1. Comparison of biochemical data between acute kidney injury and control groups

Parameters	Controls (mean±SD)	AKI (mean±SD)	p
BUN mg/dL	24.8±7.6	129.3±85.1	<0.001[a]
Creatinine mg/dL	0.9±0.1	3.0±1.5	<0.001[a]
Total protein g/dL	7.14±0.56	6.67±0.73	0.003[b]
Albumin g/dL	4.23±0.33	3.57±0.62	<0.001[a]
25-hydroxyvitamin D ng/ml	11.3±12.9	11.1±9.3	0.925
PTH pg/ml	56.9±30.6	111.1±63.7	<0.001[a]
Calcium mg/dL	9.3±0.4	8.7±0.8	0.001[c]
Corrected calcium	9.3±0.4	8.8±1.3	0.025[b]
Magnesium mg/dL	2.1±0.1	2.2±0.6	0.212
Phosphorus mg/dL	3.6±0.6	4.8±1.7	0.001[c]
GGT U/L	19.6±7	22.3±11	0.262
ALP U/L	97.6±24	92.5±17	0.303
Uric acid mg/dL	5.2±1.5	9.3±3.9	<0.001[a]
Endocan ng/L	199±56	336±289	0.006[b]

a: p<0.001; b: p<0.05; c: p=0.0001; AKI: Acute kidney injury; ALP: Alkaline phosphatase; BUN: Blood urine nitrogen; GGT: Gamma glutamyl tansferase; PTH: Parathyroid hormone; SD: Standard deviation.

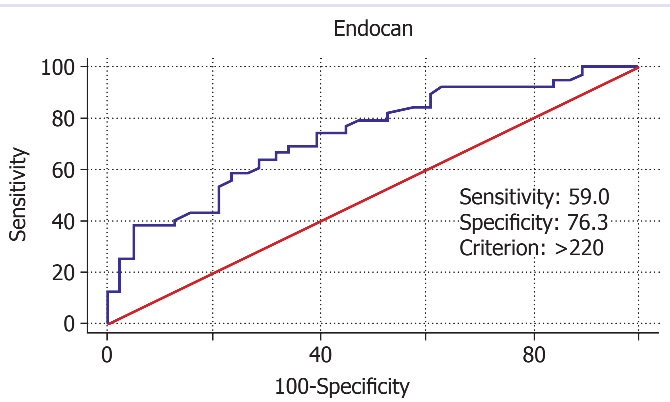

FIGURE 1. ROC analysis of serum endocan for the diagnosis of acute kidney injury.

endocan had a sensitivity of 59%, specificity of 76.3%, and AUC of 0.733 (95% CI: 0.620–0.827) for the diagnosis of AKI (Figure 1).

DISCUSSION

The results of this study revealed a higher endocan level in patients diagnosed with AKI when compared to the healthy individuals. However, vitamin D deficiency was observed in both groups. This clearly demonstrates the role of inflammation and endothelial dysfunction in AKI etiopathogenesis. Furthermore, the ROC analysis confirmed the high sensitivity and specificity of endocan (59% and 76.3%, respectively) in the diagnosis of AKI. However, since these values were obtained against a healthy control group and considering the increased endocan levels in the presence of several other diseases, we suggest that the specificity of endocan might be lower and therefore the physicians should take care when performing a differential diagnosis of AKI.

It is not yet clear whether the increased endocan level in patients with kidney diseases is due to elevated secretion or a reduced clearance of ESM-1. The clearance mechanism of endocan has also not been clarified. Therefore, in this study, the increased level of endocan found in in cases with reduced kidney function may be associated with either increased production or reduced clearance of this protein [6].

Su et al. reported a correlation between serum endocan levels and chronic kidney disease, and associated the increased endocan levels with the progression of graft renal dysfunction. In the same study, the researchers found a correlation between TNF-α, an inflammatory cytokine known to stimulate endothelial cell activation and damage, and serum endocan [17]. In a similar study conducted with patients that had undergone renal transplantation, endocan was reported to be a sensitive and specific marker of acute rejection, indicating the degree of cell damage in renal allografts [18].

Yilmaz et al. investigated the plasma endocan level of patients with chronic kidney disease and found it to be correlated negatively with the estimated glomerular filtration rate (eGFR) and positively with pentraxin 3 and high-sensitivity C-reactive protein (hsCRP), markers of inflammation. Furthermore, the authors used two vascular abnormalities of flow-mediated vasodilatation and carotid intima–media thickness to assess endothelial dysfunction and demonstrate their association with endocan. In addition, one of the most important findings was presented as the association between elevated plasma endocan level and the increased risk of cardiovascular diseases and all-cause mortality [7].

Serum endocan level has been reported to be higher in hypertensive patients, and attributed to its association with carotid intima–media thickness and hsCRP. This relationship has been attributed to endothelial dysfunction and inflammation [19].

A high plasma endocan level has been suggested as a prognostic marker of immunoglobulin A nephropathy through demonstrating advanced pathologic grades and a rapid decline in eGFR [20]. Chen et al. found the inflammatory response of ischemic AKI to be associated with increased expression of the adhesion molecules of the endothelium [21]. All these results are consistent with those reported in the present study.

In this study, the vitamin D levels of the study and control groups were similar, both indicating a deficiency. A 25(OH)D level of 20 ng/mL indicates vitamin D deficiency, 21 to 29 ng/mL refers to insufficiency, a value higher than 30 ng/mL represents a sufficient level (preferred range: 40–60 ng/mL) and above 150 ng/mL is considered to be vitamin D intoxication [9].

In cases of end-stage renal disease, the serum vitamin D levels have an inverse relation with endothelium-dependent dilation, vascular endothelial function, and vascular endothelial inflammation [22]. In their study, Braun et al. concluded that 25(OH)D deficiency was a significant predictor of AKI and mortality in critically an ill patient population [23]. Gonçelves et al. created an AKI rat model induced by ischemia/reperfusion damage, and demonstrated that vitamin D deficiency increased tubulointerstitial damage and the formation of interstitial fibrosis and thus aggravated the progression ofchronic kidney disease [24]. Similarly, in another experimental study on rats, the group, in which both vitamin D deficiency and ischemia/reperfusion AKI were induced, was found to experience a higher decline in renal function, having a higher excretion of urinary protein, greater renal tubular damage and lymphocyte infiltration compared to the group that was only subjected to ischemia/reperfusion AKI. Based on this result, the authors concluded that vitamin D deficiency aggravated AKI [25]. In another study, the vitamin D deficiency in AKI patients was inversely related to the severity of sepsis and mortality [26].

The results of previous research as well as the present study show that vitamin D deficiency is commonly seen in AKI patients. In this study, the absence of a significant difference between the study and control groups in terms of the vitamin D levels can be attributed to the generally low levels of vitamin D observed throughout the world for all age groups and both genders [9].

In this study, the PTH and P levels were found to be higher and Ca and albumin lower in the study group than the control group. This is also consistent with earlier reports [27, 28]. Serum uric acid was higher in the study group, which has previously been attributed to hyperuricemia and the crystalline and non-crystalline effects of uric acid increasing the risk for AKI [29].

The presents study showed no significant difference between the two groups concerning serum ALP and GGT levels. Similarly, other researchers have demonstrated that despite the increased levels of brush border enzymes in urine indicating cell necrosis, serum levels do not reflect the same change [30, 31].

In conclusion, this study is significant in terms of being the first report in the literature on the endocan levels of AKI patients. Serum endocan levels were found to increase in the presence of AKI secondary to inflammation and endothelial damage. Another significant finding is the common vitamin D deficiency among these patients.

Conflict of Interest: The authors stated that there are no conflicts of interest regarding the publication of this article.

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