

Research Article



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Serum fetuin-A levels and association with hematological parameters in chronic kidney disease and hemodialysis patients

Kronik böbrek hastalığı ve hemodiyaliz hastalarında serum fetuin-A seviyeleri ve hematolojik parametrelerle ilişkisi

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Abstract

Aim: The objective of the current study is to examine the association between serum fetuin-A concentrations and some other inflammation markers neutrophil to lymphocyte ratio (NLR), mean platelet volume (MPV) and C reactive protein (CRP) in patients with chronic kidney disease (CKD) and hemodialysis.

Methods: The study subjects are composed of healthy volunteers (n=47) and two patient groups; CKD patients (n=26) and hemodialysis patients (n=33). We measured serum glucose, urea, creatinine, total protein, albumin, sodium, potassium, calcium, phosphorus, iron, alkaline phosphatase, parathyroid hormone, ferritin and CRP levels by auto-analyzer and fetuin-A levels by ELISA method. Also, complete blood count parameters were analyzed and NLR was calculated.

Results: There were significant differences in serum fetuin-A concentrations, NLR and MPV values among

three groups ($p < 0.001$, $p < 0.001$, $p < 0.001$). The correlation analyses revealed that fetuin-A negatively correlated with urea, creatinine, ferritin, and CRP concentrations (r : 0.349, 0.367, 0.399, 0.550, respectively, $p < 0.05$).

Conclusion: Fetuin-A is lower in CKD and hemodialysis patients than the control group; supporting as a negative acute phase reactant. Determination of serum fetuin-A, NLR and MPV might be useful to assess inflammation in CKD and hemodialysis patients.

Keywords: Fetuin-A; Inflammation; Neutrophil to lymphocyte ratio; Mean platelet volume; Chronic kidney disease; Hemodialysis.

Öz

Amaç: Bu çalışmanın amacı kronik böbrek hastalığı (KBH) ve hemodiyaliz hastalarında serum Fetuin-A seviyeleri ve nötrofil lenfosit oranı (NLR), ortalama trombosit hacmi (MPV) ve C reaktif protein (CRP) gibi inflamatuvar parametreler arasındaki ilişkiyi araştırmaktır.

Gereç ve Yöntem: Çalışma grubu 47 sağlıklı gönüllü ile 26 KBH ve 33 hemodiyaliz hastadan oluşmaktadır. Bu çalışma için serum glukoz, üre, kreatinin, total protein, albümin, sodyum, potasyum, kalsiyum, fosfor, demir, alkalin fosfataz, paratiroid hormon, ferritin ve CRP seviyeleri otoanalizör ile, fetuin-A konsantrasyonu ise ELISA metodu ile ölçüldü. Ayrıca tam kan sayımı yapıldı ve NLR hesaplandı.

Bulgular: Üç grup arasında Fetuin A, NLR ve MPV değerleri açısından anlamlı fark vardı ($p < 0.001$, $p < 0.001$, $p < 0.001$). Korelasyon analizinde fetuin A'nın üre, kreatinin, ferritin ve CRP ile negatif ilişkili olduğu görüldü (r : 0.349, 0.367, 0.399, 0.550, sırasıyla, tüm $p < 0.05$).

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Sonuç: Fetuin-A KBH ve hemodiyaliz hastalarında kontrol grubuna göre daha düşük bulundu. Bu da negatif akut faz reaktanı olduğunu destekliyordu. NLR ve MPV değerleri kontrol grubuna göre KBH grubunda yüksekti. Fetuin A, NLR, MPV değerlerinin KBH ve hemodiyaliz hastalarında belirlenmesi inflamasyonun değerlendirilmesinde yararlı olabilir.

Anahtar Kelimeler: Fetuin-A; İnflamasyon; Nötrofil lenfosit oranı; Ortalama trombosit hacmi; Kronik böbrek hastalığı; Hemodiyaliz.

Introduction

Chronic kidney disease (CKD), includes conditions that destroy the kidney and causes loss of its functions. The incidence of CKD has been increasing dramatically for years. CKD resulted in 956,000 deaths in 2013 in the USA [1]. So, CKD is emerging as a major public health problem. CKD progresses slowly over a long period of time and takes months or years. Diabetes is the most common cause of CKD. The other causes are hypertension, glomerulonephritis, polycystic kidney disease etc. CKD may result in renal failure if untreated. End stage renal disease (ESRD) is the most prominent consequence of CKD [2]. The patients with ESRD have been forced to live depending on hemodialysis.

Fetuin-A, also known as α -2 hermanschemid glycoprotein belongs to the cystatin super family, a cluster of cysteine protease inhibitors. It is mainly synthesized by the liver and secreted into the bloodstream [3]. Its production is closely regulated by the inflammatory status of the body. Inflammation decreases the fetuin-A secretion, so it is accepted as a negative acute phase protein [4]. It is revealed that its levels decrease in acute inflammation, trauma, and bacterial infections [5–7]. It shows a negative correlation with the levels of proinflammatory cytokines such as TNF, IL-6, and IFN- γ [5, 8]. In CKD there is continuing inflammatory milieu. As a result of this conditioning serum fetuin-A levels are lower in CKD patients [9]. In addition to its role in inflammation, it has several biological functions such as, inhibiting hydroxyapatite formation, having neuroprotective effects [10], preventing vascular calcification, taking a role in atherosclerosis [11], and inhibiting insulin receptor tyrosine kinase activity [12].

Platelets play a pivotal role in inflammation by secreting many proinflammatory cytokines and inducing leukocytes to move to the inflammation site. Mean platelet volume (MPV) is a quantity of the average size of platelets in a blood sample is a kind of indicator of platelet function

and activation and is attracting increasing notice in recent years. It is demonstrated that MPV levels are associated with prediabetes, diabetes, obesity, acute coronary syndrome paroxysmal atrial fibrillation, the risk of thrombosis, low and high degree inflammatory diseases, and cerebrovascular morbidity [13–18].

Neutrophil to lymphocyte ratio (NLR) is also a potential marker to determine the inflammation. It is a simple and inexpensive marker of systemic inflammatory response. There are many studies that investigated NLR in plenty of inflammatory based diseases (diabetes mellitus, coronary artery diseases, chronic renal diseases, malignancies, Alzheimer's disease, fibromyalgia, inflammatory arthritis, metabolic syndrome, ulcerative colitis) [19–24].

In literature, there are many studies about fetuin-A levels in patients with CKD and hemodialysis. The majority of these studies were designed to reveal the inhibitory effects of fetuin-A on systemic calcification. Moreover, they mainly investigated the relation of fetuin-A on mortality, vascular calcifications, atherosclerosis, and cardiovascular risk factors [25, 26]. However, there is not any study about the relation of fetuin-A with inflammatory markers such as MPV, NLR, and ferritin in patients with CKD and on chronic hemodialysis.

In the present study, we aimed to evaluate the association between fetuin-A and some inflammatory markers and hematological parameters in patients with CKD and ESRD on hemodialysis.

Materials and methods

Study participants

A total of 106 subjects contributed in this study; 47 healthy volunteers, 26 patients with CKD and 33 with ESRD and on hemodialysis (HD). The CKD group was consisted of stage 2 (n=2, 7.7%); stage 3a (n=10, 38.5%); stage 3b (n=7, 27%); stage 4 (n=5, 19%) and stage 5 (n=2, 7.7%) patients. Exclusion criteria for patients were: lactation, pregnancy, hypothyroidism, lower extremity varicose, inflammatory diseases, alcoholism, general malignancies, chronic hepatitis, cirrhosis of the liver, bronchial asthma, high levels of hyperlipidemia, chronic obstructive pulmonary disease, collagen diseases, persistent transaminase elevation, and local or systemic infections.

Local ethical committee approved the study protocol and signed informed consent forms were obtained from all participants.

Samples and laboratory measurements

Venous blood samples were collected from patients in the morning after 12 h of the fasting period and centrifuged at 3000 g for 15 min to obtain serum. Blood sampling was performed before hemodialysis session in patients with ESRD group. Serum glucose, urea, creatinine, total protein, albumin, sodium, potassium, calcium, phosphorus, iron, unsaturated iron-binding capacity (UIBC), alkaline phosphatase (ALP), parathyroid hormone (PTH), ferritin, C reactive protein (CRP), and HbA_{1c} were analyzed by Roche Cobas Integra 8000 (Germany) auto-analyzer with Roche Cobas reagent kits (Germany); complete blood count was analyzed by Abbott Cell Dynanalyser (IL, USA) at the same day. TIBC was calculated from the sum of serum iron and unsaturated iron-binding capacity (UIBC). The supernatants of sera samples were aliquoted in microcentrifuge tubes and stored at -80°C until laboratory testing.

Serum fetuin-A levels were determined by the enzyme-linked immunosorbent assay (ELISA) with a commercial kit (BioVendor, Czech Republic) according to the manufacturer's instructions. The intra-assay coefficient of variations (CV) for fetuin-A kit were 2.2 and 3.6% at the concentrations 282.2 and 373 µg/mL, respectively. Inter assay CVs were 3.1 and 6.3% at the concentrations 266 and 314.9 µg/mL, respectively. Measurements were taken using ELISA plate reader Thermo Multiscan Go and Thermo Scientific plate washer (Thermo Fisher Scientific Inc., USA). The absorbance was measured at 450 nm in a microplate reader.

Statistical analysis

All the statistical analyses were conducted with the SPSS software (Version 16.0 SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to summarize the data. The quantitative variables were investigated using the Kolmogorov-Smirnov test to determine whether or not they are normally distributed. Between-group comparisons were assessed for nominal variables with the χ^2 -test. Differences between groups were analyzed using "One-way ANOVA" (parametric distributed variables) and "Kruskal-Wallis and Bonferroni corrected Mann Whitney U" (nonparametric distributed variables) tests. Pearson and Spearman correlation coefficient was used to determine correlations between variables. To identify the accuracy and respective best cut-off values of fetuin-A for foreseeing in CKD patients, receiver operating characteristics (ROC) curve and their comparable area under the

curve (AUC) were utilized. A p-value <0.05 was considered statistically significant.

Results

Table 1 shows a comparison of the serum levels of fetuin-A, inflammatory markers and biochemical parameters between study groups. As shown in the table and Figure 1A the mean serum concentration of fetuin-A was significantly lower in the CKD and HD group than the control group ($p < 0.001$). Serum total protein ($p < 0.01$), albumin ($p < 0.001$), calcium ($p < 0.01$) concentration and TIBC ($p < 0.01$) of the CKD group were significantly lower than the control group. While there was a significant difference between CKD and control group ($p < 0.01$) according to NLR values, the difference is not significant between HD and control group ($p > 0.05$) (Figure 1C). Serum urea ($p < 0.001$), creatinine ($p < 0.001$), PTH ($p < 0.01$) concentrations, and HbA_{1c} ($p < 0.01$), MPV ($p < 0.001$) values (Figure 1B) were significantly higher in CKD group comparing with the control group. Serum urea, creatinine, PTH, ferritin, CRP concentrations, and MPV values (Figure 1B) were significantly higher in the HD group comparing with the control group ($p < 0.001$).

Comparing HD group with CKD group; urea, creatinine, potassium, phosphate, iron, ALP, PTH, ferritin and CRP were high in HD group ($p < 0.001$), whereas TIBC ($p < 0.01$), Hemoglobin ($p < 0.001$) and MPV ($p < 0.001$) were low in HD group. HbA_{1c} values were significantly high in CKD group ($p < 0.01$) comparing with the control group, however, there was no significant difference between CKD-HD groups and HD-control groups ($p > 0.05$).

The correlation analyses examined in all groups revealed that fetuin-A negatively correlated with urea, creatinine, ferritin, and CRP concentrations (r : 0.349, 0.367, 0.399, 0.550, respectively, $p < 0.05$) and positively correlated with total protein, calcium, iron concentrations and TIBC levels (r : 0.388, 0.349, 0.254, 0.437, respectively, $p < 0.05$). In addition, the correlation analyses examined in the CKD group showed only one significant correlation that fetuin-A negatively correlated with CRP (r : 0.659, $p < 0.05$). There was no significant correlation in the HD group in any parameters.

The ROC curve which has the true-positive rate (sensitivity) on the vertical axis and the false-positive rate (1-specificity) on the horizontal axis was used to determine the potential of fetuin-A and for discriminating the control group from the patient groups. The sensitivity and specificity of fetuin-A for predicting the existence of the disease were 86% and 58%, respectively (Figure 2). The

Table 1: Comparison of the serum levels of fetuin-A, inflammatory markers and biochemical parameters between study groups.

	Control (n=47)	CKD (n=26)	HD (n=33)
Age, years	52.00±12.55	70.62±11.37 ^a	55.88±16.17 ^b
Fetuin-A, g/L	0.31±0.04	0.26±0.07 ^a	0.25±0.04 ^a
Glucose, mg/dL	96.9±14.1	108.92±34.98	126.03±67.58
Urea, mg/dL	31.38±6.63	68.9±39.81 ^a	122.82±28.27 ^{a,b}
Creatinine, mg/dL	0.9±0.12	1.86±0.81 ^a	6.87±2.35 ^{a,b}
Total protein, g/dL	7.68±0.49	6.9±0.89 ^a	7.31±0.62
Albumin, g/dL	4.19±0.25	3.51±0.57 ^a	5.09±0.75 ^{a,b}
Sodium, mEq/L	137.9±1.25	136.38±3.25	136±4.76 ^a
Potassium, mEq/L	4.23±0.3	4.14±0.5	4.99±0.89 ^{a,b}
Calcium, mg/dL	9.89±0.37	9.18±0.83 ^a	9.4±1
Phosphorus, mg/dL	3.12±0.68	3.58±0.87	6.03±2.02 ^{a,b}
Iron, µg/dL	72.77±36.26	51.77±38.95	87.91±35.01 ^b
TIBC, µg/dL	292.43±66.64	227.42±67.64 ^a	177.45±43.98 ^{a,b}
ALP, U/L	85.76±23.17	75.04±23.68	277.18±100.71 ^{a,b}
PTH, pg/mL	44.53±26.15	192.91±145.94 ^a	478.01±345.64 ^{a,b}
Ferritin, ng/mL	129.43±90.74	243.17±217.84	1178.94±410.03 ^{a,b}
CRP, mg/dL	0.73±0.96	3.91±6.37 ^a	11.86±11.01 ^{a,b}
HbA _{1c} , %	5.79±0.51	6.73±1.9 ^a	6.21±0.88
NLR	2.02±0.75	3.09±2.26 ^a	2.77±2.62
MPV, fL	9.93±0.98	11.1±0.91 ^a	8.08±0.98 ^{a,b}

TIBC, total iron binding capacity; ALP, alkaline phosphatase; PTH, parathyroid hormone; CRP, C-reactive protein; NLR, neutrophil to lymphocyte ratio; MPV, mean platelet volume; HbA_{1c}, glycated hemoglobin.

^ap < 0.05. Significant change with respect to the control group; ^bp < 0.05. Significant change with respect to the CKD group; parameters are shown as mean ± SD.

AUC was 0.706. The positive and negative predictive value of fetuin-A were 33% and 94%, respectively.

Discussion

In this study, we analyzed some biochemical parameters, inflammatory markers and serum fetuin-A levels in patients with CKD and HD and compared them with the control group. We found a significant difference in serum fetuin-A concentrations, NLR and MPV values among the three groups. The correlation analyses revealed that fetuin-A negatively correlated with urea, creatinine, PTH, ferritin, and CRP concentrations.

In CKD, inflammation is pervasive and it contributes to the morbidity and mortality. Disturbances in renal function cause uremia in patients with CKD. Uremic state causes a tendency to infection and various abnormalities in the immune system. HD patients have similar situations. Repeated dialysis causes leukocyte activation and causes chronic inflammation by producing many cytokines [27]. In the case of this inflammation while levels of some protein increases (positive acute phase proteins), some of them decreases (negative acute phase proteins). It is suggested that metabolic and hemodynamic abnormalities are the major causative factors of

renal damage, especially in diabetic patients. However, some researchers revealed that inflammation and oxidative stress play a pivotal role in the development and progression of CKD [28–30]. CKD and HD patients have a high prevalence of inflammation and oxidative stress, and these two conditions often occur concomitantly. It is claimed that inflammation provokes oxidative stress and increased oxidative stress, in turn, induces inflammation by activating nuclear transcription factor kB (NF-kB) [31]. Repeated contact of leukocytes with dialysis apparatuses such as tubes, membranes, and admixtures in dialysis water and/or dialysis solution lead to chronic inflammation. Chronic inflammation increases oxidative and carbonyl stress, in turn, they increase release and decrease the clearance of inflammatory cytokines.

Our results showed that HD patients have higher serum ferritin, and CRP levels compared with other groups. CRP and ferritin are positive acute phase proteins. The higher CRP and ferritin levels designate that there is an underlying inflammation which induces their secretion from the liver. So this finding shows that HD patients have the highest inflammatory milieu compared with CKD and control group. Serum urea levels were higher in CKD group and highest in HD patients compared with the control group as expected. Uremic state triggers inflammatory reactions and induces the secretion

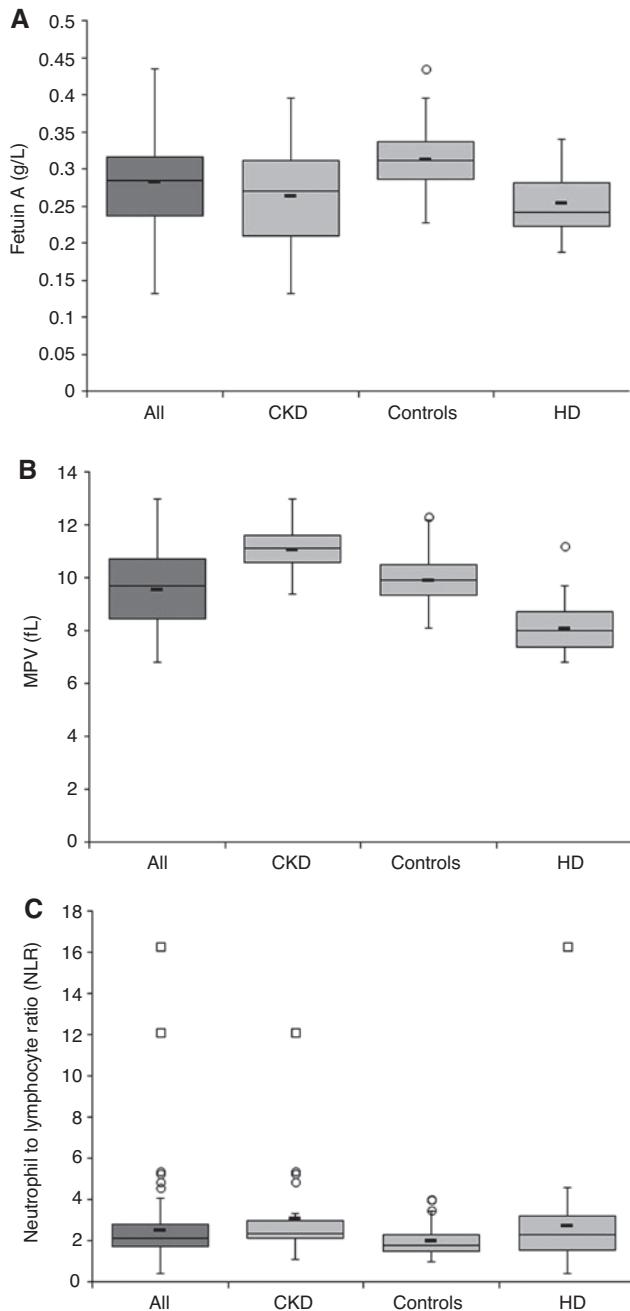


Figure 1: (A) Box-plot graphics of fetuin-A levels in patients with CKD, hemodialysis and controls. (B) Box-plot graphics of MPV (fL) levels in patients with CKD, hemodialysis and controls. (C) Box-plot graphics of neutrophil to lymphocyte ratio (NLR) levels in patients with CKD, hemodialysis and controls.

of many cytokines. Concordant with our results Thahrity et al. reported that HD patients had higher levels of pro-inflammatory cytokines such as $\text{TNF-}\alpha$, $\text{IL-1}\beta$ and IL-6 , CRP, fibrinogen, and ferritin levels compared with chronic renal failure (CRF) patients [32].

Activated platelets play crucial roles in inflammation. Platelets can interact with many inflammatory cells

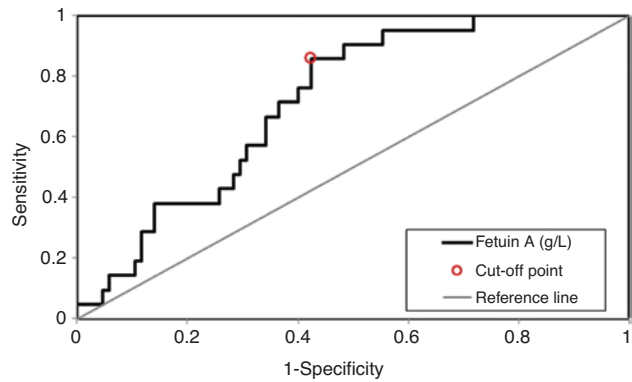


Figure 2: Receiver operating characteristics (ROC) curve for serum fetuin A levels.

such as lymphocytes, neutrophils, and phagocytes. These interactions trigger inflammation, leads to cytokine production and leukocyte chemotaxis [33]. MPV reflects the activity of platelets. Larger platelets are metabolically and enzymatically more active than the smaller platelets and they secrete greater quantities of thromboxane A_2 , platelet factor 4, and thromboglobulin [34]. There are some studies that investigate the relation of MPV and CKD but the results were conflicting and controversial. Ju et al. demonstrated that higher MPV was noted in patients with CKD and it had an inverse correlation with estimated glomerular filtration rate (eGFR) [35]. Discordant to that study Bilen et al. reported that there was not a significant difference between the transplanted, pre-dialysis, and dialysis patients according to MPV values [36]. Our results are in concordance with both of these reports in some parts. Our data showed that CKD group had higher MPV values than the control group, but the HD patients had lower MPV values than CKD and control groups. However, they did not compare the results of patient groups with the control group, in both studies. So it is uncertain, whether MPV is significantly different in the control group according to patients with CKD.

White blood cells and their subtypes are remarkable inflammatory markers. NLR is a novel inflammatory biomarker used as a prognostic factor in various diseases. Neutrophilia and relative lymphocytopenia were suggested to be independent predictors of mortality in patients with acute heart failure [21]. Recently, we showed that NLR could be used to predict mortality during the follow-up of heart failure patients [22]. We also showed that patients with non-dipper hypertension had significantly higher NLR compared to dipper hypertension [23]. In the present study, the CKD group had significantly higher NLR than the control group. Additionally, Turkmen et al. reported that NLR was closely associated with increased

inflammation in both HD and peritoneal dialysis (PD) patients. PD patients had higher NLR than ESRD and HD patients [24].

The other main finding of the presented study was, the control group had the highest serum fetuin-A levels while the HD group the lowest. In correlation analyses, we revealed that there were inverse correlations between serum fetuin-A levels and serum ferritin and CRP levels. These findings support that fetuin-A is a negative acute phase reactant and inflammation down-regulates its expression. Our results are in accordance with the study that describes the relation of fetuin-A with cytokine concentrations in patients with chronic renal failure. They reported that HD and CKD patients had higher levels of proinflammatory cytokines and CKD patients had higher serum fetuin-A levels than HD patients. Moreover, they also found that serum fetuin-A levels were inversely related to proinflammatory cytokines such as IL-1b and CRP [37].

In our study, we found that there were negative correlations between serum fetuin-A and urea and creatinine concentrations. It shows that serum fetuin-A levels decrease with the deterioration of renal functions. In recent studies, it is shown that serum fetuin-A levels notably decreased in children with nephrotic syndrome and fractional excretion of fetuin-A was significantly associated with the degree of proteinuria [38]. In another study, it is reported that urinary levels of fetuin-A normalized to creatinine were significantly higher in autosomal dominant polycystic kidney disease (ADPKD) patients compared to healthy volunteers. They reported that a significant correlation was found between CKD stages and the levels of urinary fetuin-A in ADPKD patients. Finally, they suggested that the levels of fetuin-A in urine may be a sensitive marker in determining disease progression in ADPKD [39]. In the light of these findings, decreased levels of serum fetuin-A levels in HD patients and inverse correlation with urea and creatinine concentrations may be due to increased urinary excretion of fetuin-A or may be due to down regulation of its hepatic synthesis because of an increased uremic and inflammatory state in HD patients.

When we investigated the correlation of serum fetuin-A levels with NLR and MPV values, we did not observe any significant correlation between these parameters.

We determined cut-off values discriminating healthy volunteers from patients using the ROC curve. Serum fetuin-A levels of healthy volunteers showed the highest AUC (0.706, $p < 0.05$) and that fetuin-A values at 0.282 g/L distinguished healthy controls from patients with 86% of sensitivity and 58% of specificity.

We can conclude that there were several main findings of the present study. First inflammation markers

including CRP, ferritin, NLR, and MPV were significantly increased in CKD patients when compared to healthy volunteers. Second, CKD and HD patients had lower serum fetuin-A levels compared to healthy volunteers. Those findings support the claims that fetuin-A is a negative acute phase protein. Also, serum fetuin-A levels negatively correlated with CRP, ferritin, urea, and creatinine. Moreover, although the CKD had higher NLR and MPV levels compared to control groups, there was no significant correlation between fetuin-A and NLR and MPV values. In addition, serum fetuin-A levels can discriminate the healthy volunteers from the patients with 86% of sensitivity and 58% of specificity.

In summary, identification of serum fetuin-A, NLR and MPV might present useful information to assess inflammation in CKD and hemodialysis patients.

Conflict of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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