

# The evaluation of the relationship between fetuin-A and traditional and non-traditional cardiovascular risk factors in kidney transplantation recipients

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## ABSTRACT

**Objectives:** Kidney transplantation recipients (KTRs) have higher cardiovascular complications risk compared to the general population. Cardiovascular risk factors (CVRF, Traditional and non-traditional) are widely studied to understand the causes of increased cardiovascular disease (CVD) risk in KTRs. Fetuin-A prevents from vascular calcification (VC) by inhibiting production and collapsing of apatite crystals to the vascular wall. The relationship between fetuin-A levels and CVRF in KTRs was investigated in this study.

**Methods:** Sixty-two KTRs with no prior CVD history participated. Anthropometrical, laboratory (fetuin-A, inflammation markers, antioxidants, lipid peroxidation products) and cardiological (echocardiographic, pulse wave velocity) measurements were performed. Participants were divided into two groups according to normal ( $\geq 0.5$  g/L, n = 32, NFA) and low ( $< 0.5$  g/L, n = 30, LFA) fetuin-A levels according to manufacturer's reference range, and the results were compared.

**Results:** No significant difference was observed in demographic features, body mass index, systolic and diastolic blood pressures, left ventricle mass index, waist and hip circumferences, left ventricle hypertrophy and waist-hip ratios between the two groups ( $p > 0.05$ ). The ratios of drug usage such as immunosuppressives, anti-hypertensives and statin were comparable between two groups. Parathormone levels were significantly higher in the NFA group ( $p = 0.015$ ) and glomerular filtration rate was calculated significantly higher in LFA group ( $p = 0.007$ ). The comparison of other CVRF reveals no significant difference ( $p > 0.05$ ).

**Conclusions:** Although many CVRF improved in KTRs, subclinical inflammation markers were still higher than the healthy population. Identification and early recognition of CVRF in high-risk individuals may contribute to the reduction of cardiovascular mortality. In our study, we observed no significant relationship between fetuin-A levels and CVRFs. We evaluated the relationship between serum fetuin-A levels on cardiovascular risk factors by its role in pathophysiology.

**Keywords:** Kidney transplantation, cardiovascular risk factor, fetuin A

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The most common cause of death in dialysis patients is cardiovascular disease (CVD) [1]. Although CVD risk after a successful kidney transplantation decrease compared with waitlisted patients on dialysis, transplant patients still have high risk of vascular complications compared with the general population [1, 2]. The causes of increased CVD risk in recipients are an exacerbation of traditional risk factors [advanced age, male gender, low HDL cholesterol, diabetes mellitus, smoking, race, family history, left ventricular hypertrophy (LVH), dialysis vintage, hypertension, increased low density lipoprotein (LDL) cholesterol, menopause, low levels of physical activity] due to the effect of immunosuppressive drugs, and non-traditional risk factors [proteinuria, hyperhomocysteinemia, elevated levels of lipoprotein (a) and apolipoprotein A isoforms, anemia, abnormal calcium (Ca) and phosphorous (P) metabolism, extracellular volume overload, oxidative stress, inflammation (elevated C-reactive protein and interleukin-6 levels), malnutrition, thrombogenic factors, sleep disorders, nitric oxide and endothelin balance changes] related to immunosuppressive drugs or to chronic kidney disease (CKD) [3, 4].

Vascular calcification (VC) and inflammation are common complications contributed to the development of CVD in patients with CKD. Disturbances of Ca and P homeostasis may induce calcification processes in these patients. Other factors related with the pathogenesis of VC in CKD include increased duration of uremia, increased dialysis vintage, low serum fetuin-A level and high serum level of fibroblast growth factor 23 (FGF23) [5]. Inflammation in CKD patients may contribute to endothelial dysfunction that observed in patients even in the early stages of the disease, and accelerated atherosclerosis [6]. In addition, inflammation is associated with VC or valvular calcification in CKD patients [7]. The presence of VC in hemodialysis patients is associated with increased stiffness of large capacitative elastic-type arteries like common carotid artery and aorta [8]. Arterial stiffness (AS) is known to occur before the onset of clinical disease in CKD patients and it is an independent risk factor for CVD. Transplantation improves large and to some extent small artery elasticities. Damaged large arterial compliance has an important role in the genesis of high systolic blood pressure (BP), high pulse pressure and increased LVH, while small arterial elasticity is related

to endothelial dysfunction [9].

Fetuin-A is a glycoprotein that prevent from VC by inhibiting production and collapsing of apatite crystals to the vascular wall. Fetuin-A levels found to be very low in patients with kidney failure who have high risk for VC, and increased CVD frequency can be related with low fetuin-A levels in hemodialysis patients [10]. It is remarkable that inflammation markers related VC is seen in transplant recipients rather than traditional risk factor related AS [11]. The present study aimed to evaluate the relationship between serum fetuin-A levels with traditional and non-traditional CVD risk factors in transplant recipients.

## METHODS

The cohort included stable consecutive kidney transplant recipients who were older than 18 years and on regular outpatient clinic visits. We used the following exclusion criterias; presence of known CVD (coronary artery disease, heart failure, peripheral vascular disease, prior myocardial infarction), acute or chronic liver disease, any inflammatory disease, malignancy and history of hospitalization or surgery within the preceding 6 months. The study was carried out in accordance with Good Clinical Practice and the Declaration of Helsinki. The local ethics committee approved the study protocol and informed written consent was obtained from all individuals before they entered the study. Anamnestic information retrieved from all participants. A complete physical examination was performed to all recipients.

Normal fetuin-A serum concentrations (ninety-five percentile) range between 0.5 and 1.0 g/L with a mean value of 0.733 g/L and a standard deviation of 0.178 g/L. Kidney recipients were divided into two groups with normal ( $\geq 0.5$  g/L, n = 32, NFA) and low ( $< 0.5$  g/L, n = 30, LFA) fetuin-A levels.

## Anthropometrical Measurements and Definitions

In all patients, body weight, waist (WC) and hip circumferences (HCs) were measured. WC was measured at the umbilicus level after normal breath to the nearest 0.1 cm. HC was measured at the most salient point between the waist and the thigh. Height was measured to nearest 0.1 cm and weight measured using a digital scale to nearest 0.1 kg. Body mass

index (BMI) was calculated as weight in kilograms divided by the square of height in meters [weight/height<sup>2</sup> (kg/m<sup>2</sup>)]. The BMI was classified using the adult scheme proposed by the World Health Organization (WHO), which defines obesity as a BMI  $\geq 30$  kg/m<sup>2</sup> [12]. Abdominal obesity was defined using WC points of  $> 102$  cm for men and  $> 88$  cm for women.

### Blood Pressure Measurements

Systolic and diastolic BPs were measured by one of the study investigators using standard mercury sphygmomanometer. BP cuffs of an appropriate size for each subject were attached to the upper arms. BP was measured in the arms over the brachial arteries. BPs were measured from firstly right arm or arm without fistula in recipients with arterio-venous fistula.

### Laboratory Measurements

Venous blood samples were taken after an overnight fasting. Routine biochemical and hematological parameters were studied on the day that the blood was collected. Plasma samples were centrifuged and frozen in aliquots at  $-80$  °C until other assays were performed. Complete blood count (with CELL-DYN 3700), the levels of serum glucose, urea, creatinine, uric acid, Ca, P (by enzymatic method), total cholesterol (T-chol), triglyceride (by enzymatic hydrolyse method) and high-density lipoprotein (HDL) cholesterol (by enzymatic elimination method) were measured with Abbott kit in Aeroset device. LDL cholesterol was calculated with Friedewald formula [13].

Serum intact parathyroid hormone (iPTH) measured by immunoassay with Immulite 2000 AnalyserDpc. High-sensitive C-reactive protein (hsCRP) measured with solid phase chemiluminesans immune measurement method by Immulite 2500 kits in Immulite 2500 device. 24-hour urinary protein excretion (UPE) was measured by immunoassay (DCA 2000 system, Siemens AG, Munich, Germany). We calculated estimated glomerular filtration rate (eGFR) using 2009 the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation [14]. Bone mineral density (BMD) was measured with DEXA method (Hologic QDR-2000, Waltham, MA, USA) from femoral and lumbar regions. Anemia after transplantation defined as

hemoglobin falls to less than 11 g/dL in premenopausal females or to less than 12 g/dL in males and postmenopausal females [15]. Inflammation markers [interleukin 6 and 8 (IL-6, IL-8), tumor necrosis factor alpha (TNF- $\alpha$ ), fibronectin, transforming growth factor beta (TGF- $\beta$ )], anti-oxidant enzymes [whole blood glutathione peroxidase (Gpx), serum superoxide dismutase (SOD) and total antioxidant status (TAOC)], antioxidants (vitamin C) and lipid peroxidation products [red blood cell malondialdehyde (RBC MDA)] were measured.

Serum fetuin-A levels were measured by an enzyme-linked immunosorbent assay (ELISA) kit for human fetuin-A (BioVendor Laboratory Medicine, Brno, Czech Republic) according to manufacturer's instructions. Intra- and inter-assay coefficients of variation were both less than 5.0%. Normal serum concentrations of fetuin-A in healthy adults range between 0.5 and 1.0 g/L [16].

### Cardiac Measurements

The cardiac parameter's measurement in recipients was performed by the same physician at the lab of cardiology department. Echocardiographic measurements were done with Vivid 3 device (General Electrics, Vivid 3 echocardiography, Milwaukee, WI, USA). Left ventricle mass index (LVMI) was calculated by division of left ventricle mass calculated with Dewereux [13] formula (Left ventricle mass (g) =  $0.8 \times 1.04 \times [(LviDD + IVS + PWT)^3 - LviDD^3] + 0.6$ ) to body surface area with Mostellar formula [17] ( $\sqrt{\text{height} \times \text{weight}/3600}$ ). LVMI  $\geq 110$  g/m<sup>2</sup> and  $\geq 134$  g/m<sup>2</sup> is accepted as LVH in women and men, respectively. All AS measurements were performed on radial artery. Stroke volume, cardiac output, systemic vascular resistance impedance and large/small vascular elasticity indexes were measured with PulseWave Sensor HDI (Hypertension Diagnostics, Eagan, MN).

### Statistical Analysis

The data was analyzed using the IBM SPSS Software package of version 23.0 (IBM Corp, Armonk, NY, USA) licensed to Uludag University. Normality of variables was assessed by the Shapiro - Wilk's normality test. Categorical variables were given as number and percentage, and numerical variables as mean  $\pm$  standard deviation or median

(minimum-maximum). Continuous variables were compared using the parametric two independent samples t-test or the non-parametric Mann-Whitney test in intergroup comparisons. Chi square test and Fisher-Freeman-Halton test were used to test the differences in proportion of the categorical variables. Associations between variables were estimated using Pearson or Spearman correlation coefficients. A *p* value less than 0.05 was considered to be significant.

## RESULTS

### Characteristics of recipients

The cause of chronic kidney disease in 23 recipients was unknown. The primary disease of the rest was glomerulonephritis (*n* = 8), diabetic nephropathy (*n* = 2), hypertensive nephropathy (*n* = 11), obstructive nephropathy with vesicoureteral reflux (*n* = 6), polycystic kidney disease (*n* = 5), nephrolithiasis (*n* = 3) and rare diseases (*n* = 4). Before kidney transplantation, 42 patients were on

hemodialysis (HD), 8 patients were on peritoneal dialysis (PD), 7 patients were converted from PD to HD and 5 patients underwent preemptive transplantation. Mean dialysis durations were  $44.1 \pm 5.2$  months. All patients underwent first kidney transplantation and mean follow-up time after transplantation was  $36.9 \pm 4.8$  months. Numbers of diabetic and hypertensive recipients were 12 and 44, respectively.

There was no significant difference between demographic features of the two groups (Table 1). The ratios of drug usage such as immunosuppressives, anti-hypertensives and statin were comparable between two groups. Also, BMI, systolic and diastolic BPs, LVMI, waist and hip diameters, LVH and WHRs of the groups were similar (Table 1). LVMI was  $145.9 \pm 22.1$  g/m<sup>2</sup> (min-max: 110-187) in patients with LVH (*n* = 27) and  $107.3 \pm 19.4$  g/m<sup>2</sup> (min-max: 62.9-133.7) in patients without LVH (*n* = 35), *p* < 0.001. Family history for HT, DM, CAD and obesity was similar (Table 1).

**Table 1.** Comparison of Demographic data and traditional cardiovascular risk factors between the groups with normal and low fetuin-A levels

	Normal (n = 32)	Low (n = 30)	<i>p</i> value
Male/female ratio	13/19	16/14	0.316
Age (years)	37.9 ± 10.3	37.7 ± 11.9	0.934
Dialysis duration, [months, median (min-max)]	34.5 (0-145)	31 (0-190)	0.925
Dialysis modality, n (%)			0.480
HD	20 (62.5)	22 (73.3)	
PD	5 (15.6)	3 (10)	
HD+PD	3 (9.4)	4 (13.3)	
Pre-emptive	4 (12.5)	1 (3.3)	
Donor type (living/deceased)	19/13	18/12	0.960
Donor age, [years, median (min-max)]	50.5 (15-73)	46.5 (11-62)	0.179
Transplant duration [months, median (min-max)]	16.5 (7-147)	23 (6-157)	0.388
HT, n (%)	24 (75)	20 (66.7)	0.470
DM, n (%)	7 (21.9)	5 (16.7)	0.604
CAD, n (%)	-	1 (3)	0.298
Obesity, n (%)	3 (9.4)	8 (26.7)	0.075
Abdominal obesity, n (%)	13 (40.6)	16 (53.3)	0.316
Smoker (n, %)	2 (6.3)	4 (13.3)	0.346
Physical activity, n (%)	15 (46.9)	20 (66.7)	0.116

**Table 1 Continued.**

<b>Medication History</b>			
Immunosuppressive regimens, n (%)			0.167
Tac+MMF+P	18 (56.3)	17 (56.7)	
CsA+MMF+P	5 (15.5)	8 (26.7)	
Tac+AZA+P	-	2 (6.7)	
CsA+AZA+P	2 (6.3)	-	
Tac+P	-	2 (6.7)	
EVR+CsA+P	1 (3.1)	-	
EVR+Tac+P	3 (9.4)	1 (3.3)	
EVR+MMF+P	1 (3.1)	-	
SRL+MMF+P	2 (6.3)	-	
Statin use, n (%)	6 (18.7)	4 (13.3)	0.409
Anti-hypertensive, n (%)			
None	8 (25)	10 (33)	0.470
Combination	16 (50)	14 (46)	0.793
ACEI or ARB	9 (28)	7 (23)	0.667
$\beta$ -Blocker	15 (46)	7 (23)	0.053
$\alpha$ -Blocker	1 (3)	5 (16)	0.071
CaCB	19 (59)	16 (53)	0.632
Family history, n (%)			
HT	10 (31.3)	7 (23.3)	0.485
DM	7 (21.9)	5 (16.7)	0.604
CAD	8 (25)	5 (16.7)	0.421
Obesity	6 (18.8)	9 (30)	0.301
<b>Measurements</b>			
BMI (kg/m <sup>2</sup> )	25.3 $\pm$ 3.6	26.5 $\pm$ 5.4	0.295
Systolic BP (mmHg)	136.2 $\pm$ 18.1	133.1 $\pm$ 15.8	0.485
Diastolic BP (mmHg)	81.3 $\pm$ 11.2	78.2 $\pm$ 12.2	0.300
Waist circumference (cm)	93.2 $\pm$ 10.2	95.0 $\pm$ 14.1	0.570
Hip circumference (cm)	101.5 $\pm$ 9.5	105.7 $\pm$ 11.6	0.127
Waist to hip ratio	0.91 $\pm$ 0.07	0.89 $\pm$ 0.06	0.204

HD = hemodialysis, PD = peritoneal dialysis, Tac = tacrolimus, MMF = mycophenolate mofetil or sodium, P = prednisolone, CsA = cyclosporine, AZA = azathioprine, EVR = everolimus, SRL = sirolimus, ACEI = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker, CaCB = calcium channel blocker HT = hypertension, DM = diabetes mellitus, CAD = coronary artery disease, BMI = body mass index, BP = blood pressure

## Treatments

All patients were treated with tacrolimus (Tac, 0.05-0.1 mg/kg/d, n = 43) or cyclosporine A (CsA, 6 mg/kg/d, n = 16) or mammalian target of rapamycin (mTOR) (everolimus, n = 6 or sirolimus, n = 2) combined mycophenolic acid (1440 mg/d) or mycophenolate mofetil (2 g/d) (n = 51) or azathioprine (100 mg/d, n = 4) and corticosteroids

(methylprednisolone, 500 mg intravenous; then, prednisolone, 1 mg/kg/d oral). All patients received induction treatment with basiliximab (20 mg, before and 4 days after transplantation). The dosages of Tac and CsA were adjusted to achieve target trough levels (CsA: first 3 mo, 200-300 ng/mL; subsequently, 100-200 ng/mL and Tac: first 3 mo, 8-12 ng/mL; subsequently, 5-8 ng/mL). Oral prednisolone dosage

was tapered to 20 mg/d after 1 month, 10 mg/d after 2 months and 5 mg/d after 6 months.

Some recipients received antihypertensive monotherapy (n = 14) or combination therapy (n = 30) (calcium channel blockers, n = 35; beta-blockers, n = 22; renin-angiotensin-aldosterone-system blockers, n = 16 and alpha-blockers, n = 8). Other medications were insulin therapy (n = 8), oral anti-glycemic agents (n = 4), statin (n = 10), vitamin D (n = 13), bisphosphonate (n = 5) and oral calcium replacement (n = 4).

While the mean serum creatinine ( $1.52 \pm 0.6$  vs.  $1.29 \pm 0.4$  mg/dL,  $p = 0.112$ ) and UPE ( $0.44 \pm 0.83$  vs.  $0.52 \pm 1.23$  gr/day,  $p = 0.754$ ) values of groups with normal and low fetuin-A level did not differ, eGFR ( $54.8 \pm 15.3$  vs.  $67.9 \pm 21.1$  ml/min/m<sup>2</sup>,  $p = 0.007$ ) levels are higher in low fetuin A group (Table 2).

No significant differences were observed among two groups in Ca and P levels, Ca×P product and normal/pathological DEXA measurement rates. Serum PTH levels are significantly higher in normal fetuin A group ( $167.1 \pm 128.1$  pg/mL vs.  $95.2 \pm 47.86$  pg/mL,  $p = 0.015$ ). AS (large and small vessel elasticity indexes and total vascular impedance measurements),

inflammatory (hsCRP, fibrinogen, fibronectin, TNF-α, IL-6, IL-8) and oxidative stress (SOD, MDA) parameters shows no significant differences between the two groups (Table 3).

In the study group, fetuin-A levels were positively correlated with creatinine ( $r = 0.312$ ,  $p = 0.013$ ) and negatively with eGFR ( $r = -0.299$ ,  $p = 0.018$ ). There was no correlation between fetuin-A level and other parameters.

## DISCUSSION

Serum fetuin-A levels are significantly increased after successful kidney transplantation compared to CKD patients, but it is still lower than healthy population [18]. Kidney transplantation improves vascular function [19], but early cardiovascular death risk remains increased [20]. In order to reduce CV mortality, it is crucial to understand the clues for progressing CV diseases. For this purpose, traditional and non-traditional cardiovascular risk factors are defined as mentioned in introduction section.

Increased fetuin-A levels after transplantation thought that there is a relationship between low fetuin-

**Table 2.** Comparison of laboratory results in two groups with normal and low fetuin-A levels

	Normal (n = 32)	Low (n = 30)	p value
Hemoglobin (g/dL)	12.7 ± 2.0	12.9 ± 2.1	0.722
Urea [mg/dL, median (min-max)]	41 (15-119)	38.5 (23-87)	0.307
Creatinine [mg/dL, median (min-max)]	1.32 (0.94-3.46)	1.27 (0.6-2.81)	0.112
eGFR (mL/min/m <sup>2</sup> )	54.8 ± 15.3	67.9 ± 21.1	<b>0.007</b>
Uric acid [mg/dL, median (min-max)]	6.1 (2.9-9.2)	5.7 (3.2-10)	0.578
Glucose (mg/dL)	97.1 ± 28.2	87.9 ± 13.9	0.110
T-chol (mg/dL)	199.0 ± 48.1	203.0 ± 44.7	0.740
HDL (mg/dL)	52.6 ± 14.9	49.7 ± 19	0.507
LDL (mg/dL)	115.4 ± 36.9	122.1 ± 33.5	0.456
Triglyceride (mg/dL)	147 (64-390)	134 (84-346)	0.916
LVMI (g/m <sup>2</sup> )	127.8 ± 27.9	120.2 ± 28.2	0.295
LVH, n (%)	16 (50)	10 (33.3)	0.184
UPE [g/day, median (min-max)]	0.170 (0.01-3.35)	0.027 (0.01-4.87)	0.754

eGFR = estimated glomerular filtration rate, UPE = urinary protein excretion, T-chol = total cholesterol, HDL = high density lipoprotein, LDL = low density lipoprotein, LVMI = left ventricle mass index, LVH = left ventricle hypertrophy

**Table 3.** Comparison of non-traditional cardiovascular risk factors between the groups with normal and low fetuin-A levels

	Normal (n = 32)	Low (n = 30)	p value
Fetuin-A (g/L)	0.616 (0.511-1.079)	0.406 (0.153-0.497)	< <b>0.001</b>
Proteinuria, n (%)	7 (21.9)	5 (16.7)	0.751
Anemia, n (%)	9 (28.1)	5 (16.7)	0.367
Calcium (mg/dL)	9.42 ± 0.71	9.41 ± 0.57	0.927
Phosphorus (mg/dL)	3.20 ± 0.68	3.18 ± 0.67	0.880
Ca×P	30.03 ± 5.71	29.79 ± 5.82	0.872
PTH [pg/mL, median (min-max)]	113.2 (33.8-522)	83.9 (28-207)	<b>0.015</b>
Osteoporosis, n (%)	7 (21.9)	4 (13.3)	0.511
Osteopenia, n (%)	17 (53.1)	21 (70)	0.173
C1 (ml/mmHg×10)	12.75 ± 5.70	13.57 ± 4.99	0.565
C2 [ml/mmHg×10, median (min-max)]	3.9 (2.5-7.9)	5.2 (1.9-10.2)	0.185
TVI, [median (min-max)]	141.5 (58-295)	125 (79-277)	0.477
hsCRP [mg/L, median (min-max)]	2.55 (0.2-45)	2.1 (0.2-30.9)	0.689
Fibrinogen (mg/dL)	4.26 ± 1.24	4.19 ± 1.23	0.888
Fibronectine (µg/mL)	112.2 ± 25.6	115.3 ± 27.3	0.644
TNF-α [ng/mL, median (min-max)]	0.081 (0-0.53)	0.050 (0.02-0.5)	0.557
IL-6 [ng/mL, median (min-max)]	1.04 (0.76-44.8)	0.89 (0.77-23.5)	0.523
IL-8 [ng/mL, median (min-max)]	6.34 (0.4-71.9)	6.71 (0.4-33.1)	0.791
TGF-β [pg/mL, median (min-max)]	4913 (917-47651)	5363 (974-29643)	0.817
SOD [U/mL, median (min-max)]	977 (408-3059)	1140 (565-2178)	0.613
GSHpx [U/mL, median (min-max)]	18.4 (9.75-40.5)	15.8 (6.46-56.8)	0.987
MDA [nmol/mL, median (min-max)]	1.04 (0.15-3.52)	1.05 (0.38-2.67)	0.986
Vitamin C [mg/dL, median (min-max)]	2.07 (0.17-12.7)	2.18 (0.51-15.2)	0.234
TAOC [mmol/L, median (min-max)]	1.15 (0.48-5.56)	1.07 (0.43-6.37)	0.768

Ca×P = calcium × phosphorus, PTH = parathormone, DEXA = Bone mineral density, C1 = large vessel elasticity index, C2 = small vessel elasticity index, TVI = total vascular impedance, hsCRP = high sensitive C-reactive protein, TNF-α = tumor necrosis factor alpha, IL-6 = interleukin 6, IL-8 = interleukin 8, TGF-β1 = transforming growth factor β1, SOD = superoxide dismutase, GSHpx = glutathione peroxidase, TAOC = total antioxidant status, MDA = malonyl dialdehyde

A levels and glomerular dysfunction [18]. Another study in patients with coronary artery disease reported no relationship between GFR and fetuin-A concentrations [21]. Impaired endothelial functions in transplanted patients were reported in several studies [22-24]. Our data shows that patients with lower fetuin A concentration has greater eGFR ( $p = 0.007$ ), but urea and creatinine levels were similar (Table 2). Several factors may be responsible for the

discrepancies including the effect of calcineurin inhibitors [23].

In our cohort, there was no relationship between fetuin-A levels and cholesterol levels, diabetes mellitus and obesity and waist to hip ratios as other studies revealing fetuin-A levels did not correlated renal and metabolic parameters [25].

Cardiovascular events and all-cause mortality rates were significantly increased in transplant

recipients with low serum fetuin-A levels [26]. The main lesions underlying and ultimately responsible for the clinical manifestations of CVD are atheroma and VC, which often exist together [27]. Marechal *et al.* [26] have pointed out the difference in the determination of coronary artery and aortic calcification by fetuin-A levels in a multiple linear regression analysis. Although lower serum fetuin-A level was reported as an independent predictor of aortic calcification ( $p = 0.008$ ) no relationship was found in coronary artery calcification [26]. In our study, the number of patients with the history of defined coronary artery disease in the groups with low and normal fetuin-A levels were similar (Table 1).

It is reported that although, coronary artery calcification (CAC) progress is lower in transplanted patient compared with HD patients, but progression still exist. This data explained with the calcification burden during the pre-transplant dialysis period [28, 29]. No relationship between pre-transplant dialysis duration, dialysis modality, transplant duration and fetuin-A levels was observed in our study.

Diabetes mellitus is a risk factor for the presence of CAC in kidney transplant patients but has not been independently associated with CAC progression [27]. The relationship with diabetes mellitus existence and fetuin-A levels were not statistically significant in our study.

Serum fetuin-A levels are determined by variants in the plasma cholesterol levels, and a history of smoking, independently of inflammation [26]. Our data showed no difference in cholesterol levels and smoking rates depending on fetuin-A levels.

Kidney transplantation restores endothelial functions. Some studies reported impaired endothelial functions in kidney transplant recipients compared to healthy controls [22-24] which attributed to the effect of calcineurin inhibitors [23]. Inducible nitric oxide inhibition with steroids and calcineurin inhibitors that cause endothelial dysfunction, associated with onset and progression of atherosclerosis and VC. While mycophenolate mofetil has a more favorable effect on endothelial activity than do calcineurin inhibitors and steroids [30], vascular effects of mTOR inhibitors are much more complex. Rapamycin inhibits smooth muscle cell proliferation, while everolimus impairs the vasoactive and antithrombotic function of endothelial cells. In particular, sirolimus inhibits osteoclast

formation, unlike steroids and cyclosporine [31]. Our results showed no relationship between fetuin levels and immunosuppressive protocol regimens given. Hypothetical effect of immunosuppressive therapy on VC could be mediated indirectly, by interfering with post-transplant bone loss, and directly through effects that the same drugs exert on vascular cells (endothelial cells, vascular smooth muscle cells) through intermediation of the RANKL, RANK, and OPG systems [27]. Since osteoporosis rate was higher in normal fetuin A group (7 [21.9%] vs 4 [13.3%],  $p = 0.511$ ) and osteopenia rate was higher in low fetuin A group (21 [70%] vs 17 [53.1%],  $p = 0.173$ ), both results could not reach statistically significant difference.

Fetuin-A inhibits Ca-P precipitation in the serum [32]. An analysis of data obtained from 40,000 patients [33] shows that relative death risk increased 1.5 times in patients whom CaxP is 70 to 75  $\text{mg}^2/\text{dL}^2$ , and 1.8 times with a  $\text{Ca} \times \text{P} > 80 \text{ mg}^2/\text{dL}^2$ . A significant increase in mortality risk was associated with even relatively small rises in  $\text{Ca} \times \text{P}$  to 50 to 55  $\text{mg}^2/\text{dL}^2$  [33]. In our study  $\text{Ca} \times \text{P}$  values were similar in both groups.

Progression of CAC slows down but does not halt after kidney transplantation. There is a strong association between baseline CAC score and CAC progression. A significant improvement in secondary hyperparathyroidism after transplantation favorably affects the progression of CAC. Low 25(OH)D3 levels are an independent determinant of CAC progression [27]. Cianciolo *et al.* [27] reported a significant improvement in secondary hyperparathyroidism after transplantation that slow-down the progression of CAC. No relationship was found between PTH and VC in a VC progression speed study [34]. Sumida *et al.* [35] observed strong correlation between carotid plaque and male sex, presence of DM, PTH and VC. DM is a risk factor but has not been identified as an independent risk factor for coronary artery calcification progression [27]. The VC was found associated with the Ca and P multiplication ( $\text{Ca} \times \text{P}$ ). In our study, no significant difference was observed in Ca, P levels and CaxP between the groups. Number of patients with low bone mineral density between the groups was also similar. However, PTH levels were significantly higher in patients with normal Fetuin-A levels, no correlation was observed between Fetuin-A levels and bone mineralization parameters (Ca, P,

Ca×P, PTH, DEXA total lumbar score, DEXA total hip score).

Inflammation is an independent predictor of renal allograft loss [36, 37]. Patients with lower fetuin-A levels (< 0.47 g/L) and higher hsCRP levels (> 4.36 mg/L) had worse survival, with a risk of death and cardiovascular events 3.48 times higher compared with patients with higher fetuin-A and lower hsCRP combination [26]. Fetuin-A has significant anti-inflammatory functions such as inhibition of TGF- $\beta$ , tyrosine kinase activity, inflammatory cytokines IL-1 $\beta$ , IL-6 and TNF- $\alpha$  [38, 39]. Although fetuin-A acts as a negative acute phase reactant, no correlation was found between fetuin-A levels and markers of inflammation [26]. Our study did not show association between fetuin-A levels and inflammatory markers such as hsCRP, fibronectin, TNF- $\alpha$ , IL-6, IL-8 and TGF- $\beta$ .

Vascular cellular calcification is independently promoted by oxidative stress which shows procalcific effects over oxysterols, oxidized lipids and inflammatory cytokines [40]. It has been shown that vascular smooth muscle cells' osteochondrocytic differentiation is promoted by the classical oxidant stressor, H<sub>2</sub>O<sub>2</sub> [41]. Serum SOD, GSHpx and MDA levels were similar, TAOC and Vitamin C levels were not differ between the groups in this study.

Chronic inflammation in CKD patients leads to lower serum fetuin-A levels [42] and that serum fetuin-A showed important association with coronary, valvular calcification and inflammation in CKD patients [43]. In pediatric population serum fetuin-A levels did not identify as an independent predictor of vascular stiffness [44]. Aortic wall stiffness, as reflected by aortic pulse-wave velocity, is a strong predictor of cardiovascular events [45]. Our observation reveals no relationship between fetuin-A levels and total vascular impedance, large and small vessel elasticity indices.

## CONCLUSION

We evaluated the effect of kidney transplantation on cardiovascular risk factors and relationship of serum fetuin-A levels with cardiovascular risk factors by its place in pathophysiology in this study and we observed that although many cardiovascular risk

factors improved after kidney transplantation, subclinical inflammation markers were still higher than healthy volunteers. We found no relationship between serum fetuin-A levels and inflammatory, oxidative stress markers and AS parameters. Comparison of non-traditional risk factors that have an important place on cardiovascular illness pathophysiology in large transplanted and healthy volunteer population may contribute to increased survival after kidney transplantation by understanding underlying inflammatory process. Atherosclerosis and most of the cardiovascular risk factors progress silently. Early recognition of these risk factors and identification of high-risk individuals may contribute to the reduction of cardiovascular mortality.

## Conflict of interest

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