Prevalence of cardiovascular diseases in kidney transplant recipients and its relationship with asymmetric dimethylarginine, fibroblast growth factor-23 and multiple inflammatory markers

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Introduction and aim: Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of endothelial nitric oxide synthase, a marker of endothelial damage and progression of atherosclerosis. Research confirms the association of ADMA with an increased risk of cardiac complications and an increased risk of death, graft loss among kidney transplant recipients (KTRs). The aim of our study was to establish the significance of ADMA and FGF-23 as biomarkers of cardiovascular risk as well as predictors of graft failure and progression of chronic transplant kidney disease in comparison to CKD subjects. In addition, an analysis of the relationship between ADMA, FGF23 and cardiovascular diseases in CKD subjects and KTRs was performed. Methods and materials: The study group included 132 KTRs. The control group consisted of age- and sex-adjusted 40 individuals with clinically stable CKD. ADMA, FGF-23, hs-CRP and IL-6 were measured by the enzyme-linked immunoassay method (ELISA). Parameters of body mass composition such as fat mass, FTI, lean tissue mass, LTI, body water and overhydration were assessed by multi-frequency bioimpedance analysis (BIA). Results: Cardiovascular diseases (CVDs) were present in 31.8% of KTRs. Independent variables related to nutritional status (SGA, s-albumin), according to multivariate regression, may have an impact on the prevalence of CVD in the kidney transplant recipients’ group. Our study findings suggested a correlation between ADMA and serum albumin (r=-0.41, p<0.05), oxLDL (r=-0.42, p<0.05) and overhydration (OH%, r=0.28, p<0.05). Moreover, administration of statins and/or angiotensin-converting enzyme inhibitors was significantly related to a reduction of ADMA in KTRs. We have also identified a significant positive correlation between FGF-23 levels and inflammatory markers (hs-CRP, IL-6) and negative with overall index of renal function (eGFR-CKD EPI, eGFR-MDRD). Conclusion: Nutritional status, inflammation and endothelial dysfunction markers (ADMA, FGF-23) are considerably altered even in stable kidney transplant recipients.

Received: 11 March, 2022; revised: 15 July, 2022; accepted: 27 September, 2022; available on-line: 08 November, 2022

Keywords: ADMA, cardiovascular risk, FGF 23, inflammation, kidney transplantation

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Abbreviations: MBD, mineral and bone disorder; NO, nitric oxide; NOS, nitric oxide synthase; NS, not significant; NT, not tested; OH, overhydration; oxLDL, oxidized low-density lipoprotein; PTH, parathyroid hormone; ROS, reactive oxygen species; SDMA, symmetric dimethylarginine; SGA, subjective global assessment; TBW, total body water; TC, total cholesterol; TG, triacylglycerols; TNF-α, tumor necrosis factor α; VCA, vascular cell adhesion molecule; WBC, white blood cells

INTRODUCTION

Multiple factors, related to Chronic Kidney Disease (CKD), may contribute to endothelial dysfunction and result in the development of Cardiovascular Diseases (CVD) – the leading cause of death in kidney transplant recipients (KTR). Asymmetric dimethylarginine (ADMA), as an endogenous inhibitor of endothelial nitric oxide synthase (NOS), adversely affects the bioavailability of nitric oxide in blood vessels, which contributes to increased prevalence of cardiovascular events, as a result of enhanced proinflammatory, proaggregatory and proliferative activity in an intravascular environment. ADMA is widely regarded as a novel biomarker of the risk of endothelial cell dysfunction and progression of atherosclerosis. The turnover of arginine-methylated proteins provides monomethyl arginine (MMA), asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) (Post et al., 2021), simultaneously both ADMA and SDMA are regarded to be uremic toxins (Oliva-Damaso et al., 2019).

The enzyme, crucial for the regulation of ADMA concentration in tissues and intracellularly, is dimethylarginine dimethylaminohydrolase (DDAH), which initiates the hydrolysis of ADMA to dimethylamine (DMA) and L-citrulline (Oliva-Damaso et al., 2019). DDAH is synthesized mainly in the kidney and liver, while DDAH II in tissues with endothelial NOS expression (Oliva-Damaso et al., 2019). Elimination of ADMA due to urine excretion and metabolism by DDAH as well as a renal synthesis of DDAH partly explain the role of ADMA as a serological marker of increased cardiovascular burden in a patient with kidney impairment (Oliva-Damaso et al., 2019). High ADMA plasma concentration may also affect renal function due to multiple mechanisms, for example, related to impaired renal blood flow, glomerular hypertension or salt accumulation (Rysz et al., 2017; Oliva-Damaso et al., 2019). Moreover, high dietary sodium intake increases the urinary elimination of ADMA (Oliva-Damaso et al., 2019).

According to Post and others (Post et al., 2021) altered protein-arginine demethylation is common in KTR.
Additionally, elevated plasma methylated form of L-arginine – ADMA levels and decreased urinary ADMA and SDMA concentrations may indicate the kidney transplant recipients with a higher risk of all-cause mortality (Post et al. 2021). Research confirms the association of ADMA with an increased risk of cardiovascular morbidity and mortality (Schlesinger et al., 2016; Oliva-Damaso et al., 2019), as well as graft loss (Frenay et al., 2015) among kidney transplant recipients.

Endothelial dysfunction (ED) is the initial phase of vascular damage and atherosclerosis. Patients with renal insufficiency may develop ED as a result of various complications, also related to altered phosphate homeostasis – hyperphosphatemia and vascular calcification (Eskandari et al., 2017). Elevated concentration of serum FGF-23 may have an impact on vascular damage (Ashikaga et al., 2010; Eskandari et al., 2017) and extraskelatal calcification process (Jean et al., 2009; Eskandari et al., 2017) as well as increased mortality and levels of inflammatory markers in CKD patients (Munoz Mendoza et al., 2012; Eskandari et al., 2017).

Fibroblast growth factor-23 (FGF-23), as a hormonal regulator of phosphaturic function (urinary phosphate excretion inductive factor) and vitamin D metabolism (1,25-dihydroxyvitamin D synthesis inhibitory factor), plays a prominent role in this process (Eskandari et al., 2017; Kawabata et al., 2019). On the other hand, Klotho Protein, as well as phosphorus, calcium, 1,25-dihydroxyvitamin D and parathyroid hormone (PTH) have an impact on the secretion and physiologic function of FGF-23 (Kawabata et al., 2019).

Progressive loss of kidney function contributes to the increase of circulating FGF-23 levels, simultaneously, the inability of the kidney to adequately excrete urinary phosphate is contributing to hyperphosphatemia (Kawabata et al., 2019). However, excessive renal phosphorus wasting resulting in the hypophosphatemia is considered to be a possible result of the phosphaturic effect of high FGF-23 level in certain KTRs in the early time after kidney transplantation (KTx) (Ghorbani et al., 2020). Therefore, hyperphosphatemia is defined as a more characteristic condition for pre-transplant patients, whereas, hypophosphatemia frequently occurs after successful KTx and may affect approximately 40.0% of recipients within 1 month after the procedure (Kalantar-Zadeh et al., 2012; Ghorbani et al., 2020).

Mineral and bone disorder (MBD), one of the CKD frequent comorbid conditions, described primarily as abnormalities of mineral metabolism, bone morphology and turnover, leading to the increased fracture risk and potentially to vascular calcification, adversely affects morbidity and mortality of patients with CKD, particularly from cardiovascular events (Hiemstra et al., 2020).

Both, the direct impact of elevated concentration of serum FGF-23 on the renal fibrosis process and indirect suppression of 1,25-dihydroxyvitamin D and promotion of proinflammatory cytokines production may exacerbate kidney function and precipitate progression of CKD (Mehta et al., 2020).

AIM

The aim of our study was to establish the significance of ADMA and FGF-23 as biomarkers of cardiovascular risk as well as predictors of graft failure and progression of chronic transplant kidney disease in comparison to CKD subjects. In addition, an analysis of the relationship between ADMA, FGF23 and cardiovascular diseases in CKD subjects and KTRs was performed.

MATERIALS AND METHODS

The study group consisted of 132 clinically stable KTRs (57 women; 75 men; mean age, 51.06±13.57; age range, 20–75 years). All patients were treated in the Outpatient Transplantation Unit at the Department of Nephrology, Transplantology, and Internal Medicine, Medical University of Gdansk, Poland and the majority of them were treated with triple immunosuppressive protocols, including glucocorticosteroids. This study was approved by the bioethics committee of the Medical University of Gdansk (NKBBN/291-367/2020, NKBBN/291-437/2018).

The mean time from transplantation was 39.2±6.5 (range, 1–349) months, mean estimated glomerular filtration rate (eGFR), according to the Chronic Kidney Disease–Epidemiology Collaboration (CKD-EPI) equation, was 45.2±24.9 (range, 4.0–94.4) ml/min/1.73 m² in this study population. Other data, such as presence of CVD, diabetes mellitus, and medical treatment were extracted from the medical records.

The control group consisted of 40 age- and sex-matched individuals with clinically stable CKD stage 3–5 (eGFR CKD-EPI, range 6–54 ml/min/1.73 m²). In both groups, criteria to exclude from the study are failure of such organs as lungs, liver, presence of cancer, and acute disease currently under treatment.

Clinical characteristics

Cardiovascular diseases (CVDs) defined as hypertension and at least one additional condition – atherosclerosis, myocardial infarction, heart failure, cerebral stroke or cardiac arrhythmias, were found in 31.8% of KTRs, whereas almost 88.4% of the study population experienced hypertension. Comorbidities, such as diabetes mellitus, obesity and overweight were found in 31.0%, 20.8% and 35.4% of patients, respectively. Almost 15% of KTRs developed type 3 post-transplant diabetes. Only 7 patients received preemptive kidney transplant (5.0%), whereas 17 (12.9%) of KTRs underwent retransplantation. The majority of patients included in this study were treated with triple immunosuppressive protocols. Moreover, the most commonly prescribed immunosuppressive drugs were calcineurin inhibitors – tacrolimus (74.3%) and cyclosporine (23.8%), mycophenolate mofetil (86.6%) and glucocorticosteroids – methylprednisolone (84.8%). The remaining drugs, such as insulin, beta blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors (ACE-i) and statins were used respectively by 20.5%, 59.8%, 40.9%, 8.3% and 14.4% of KTRs.

Control group

Most CKD patients included in the control group had a history of hypertension (84.4%) and were treated with ACE inhibitors (29.6%). Lipid disorders, particularly hypercholesterolaemia, and statin administration, were found in 43.7% and diabetes mellitus was diagnosed in 28.0% of control group subjects. Three patients had a history of coronary disease, and two of them experienced a myocardial infarction. Medical conditions that contributed to renal dysfunction in both the study and control group are presented in Table 1.

Laboratory assay

In all study participants plasma samples were taken after an overnight fast and stored at ~80°C until analyzed.
The following compounds were measured in plasma or serum by an enzyme-linked immunoassay method:
- High sensitive C-reactive protein (hs-CRP, ELISA, DRG MedTek),
- Asymmetric dimethylarginine (ADMA Xpress ELISA, plasma),
- Tumor necrosis factor α (TNF-α, ELISA, Immunodiagnostik AG, serum),
- Oxidized low-density lipoprotein (oxLDL, ELISA, Immunodiagnostik AG, serum),
- Fibroblast growth factor-23 (FGF-23 Intact Human ELISA, Biomedica Poland, plasma),
- Interleukin-6 (Human IL-6 Quantikine ELISA, DRG MedTek, serum).

Serum albumin, creatinine, blood urea nitrogen (BUN), sodium, potassium, magnesium, lipidogram, and blood morphology were assessed according to routine methods in the hospital clinical laboratory.

The following anthropometric measurements were obtained:
- Body mass (kg), arm and waist circumference (cm),
- BMI estimated according to the current body mass/height²; BMI range <18.5 was considered underweight, 18.5–24.99 – normal weight, 25–30 – overweight, ≥30 – obesity.

Nutritional status was estimated by 7-SGA (7-point subjective global assessment):
- 6–7 – good nutritional status (not at risk for malnutrition),
- 3–5 – mild malnutrition (at risk for malnutrition),
- 1–2 – severe malnutrition

Parameters of body mass composition were assessed by the multi-frequency bioimpedance analysis (BIA) method with a BCM (Body Composition Monitor, Fresenius SA, Bad Homburg, Germany). Evaluated body mass composition parameters included:
- lean tissue mass (LTM) and lean tissue index (LTI),
- fat tissue mass (FAT) and fat tissue index (FTI),
- body water (TBW, total body water; ICW, intracellular water; ECW, extracellular water; E/I ratio; OH, overhydration).

The biochemical and anthropometric characteristics of the study and control group are presented in Table 2.

### Statistical Analysis

Statistical analysis was performed using Statistica 13.3 version for Windows. All data are presented as mean ± S.D. Comparisons between the groups were examined by Student’s t-test (for parametric data) and U Mann-Whitney Rank Sum Test (for non-parametric data).

Spearman’s correlation was used for a nonparametric measure of statistical dependence between 2 variables. Independent associations among variables were assessed with stepwise multiple regression analysis. For all performed analyses p<0.05 was considered as statistically significant.

| Table 1. Main causes of CKD in KTRs and control group. |
|---------------------------------------------------------|
| **KTRs** | **CG** |
| Subjects | 101 | 31 |
| Chronic glomerulonephritis | 17 | 11 |
| Chronic pyelonephritis | 12 | 0 |
| Polycystic kidney disease | 18 | 2 |
| Hypertensive nephropathy | 5 | 3 |
| Diabetic Nephropathy | 15 | 2 |
| Obstructive nephropathy | 0 | 0 |
| Unknown nephropathy | 17 | 4 |
| Schoenlein-Henoch nephropathy | 2 | 0 |
| IgA nephropathy | 5 | 2 |
| Other | 8 | 7 |
| Date unknown | 31 | 9 |

| Table 2. The biochemical and anthropometric characteristics of the study and control group. |
|-----------------------------------------------|
| **KTRs** | **Control group** |
| Sex (F/M) | 57/75 | 14/26 |
| Age (years) | 51.06±13.57 | 52.76±19.67 |
| BMI | 25.87±4.09 | 27.07±5.65 |
| BMI ≤ 25 (%) | 56.1 | 65.7 |
| Creatinine (mg/dL) | 2.00±1.60 | 2.83±1.92 |
| eGFR – CKD EPI (ml/min/1.73 m²) | 47.00±23.70 | 47.00±23.70 |
| hs-CRP (mg/L) | 4.88±4.15 | 2.83±2.37 |
| WBC (10⁹/L) | 8.70±3.19 | 7.02±3.86 |
| Haemoglobin (g/dL) | 11.67±2.56 | 11.46±2.58 |
| BUN (mg/dL) | 41.85±26.00 | 43.95±23.00 |
| Proteinuria (g/24h) | 0.44±0.53 | NT |
| Uric Acid (mg/dL) | 7.10±1.81 | NT |
| Glucose (mg/dL) | 114.58±24.20 | 413.5±14.65 |
| Albumin (g/L) | 35.40±6.21 | 34.75±5.95 |
| TC (mg/dL) | 210.65±45.29 | 148.31±77.02 |
| TG (mg/dL) | 171.40±80.24 | 108.71±40.08 |
| LDL (mg/dL) | 136.27±114.77 | 136.27±114.77 |
| HDL (mg/dL) | 46.73±10.48 | 48.00±19.67 |
| Sodium (mmol/L) | 139.36±3.23 | 139.36±3.23 |
| Potassium (mmol/L) | 4.37±0.49 | 4.63±0.51 |
| Magnesium (mg/dL) | 1.81±0.30 | NT |
| Phosphate (mg/dL) | 3.50±1.40 | 3.79±0.71 |
| Calcium (mg/dL) | 9.33±0.99 | 9.33±0.66 |

BMI, Body Mass Index; BUN, Blood Urea Nitrogen; CKD-EPI, Chronic Kidney Disease, Epidemiology Collaboration equation; hs-CRP, high sensitivity C-reactive protein; eGFR, estimated Glomerular Filtration Rate; HDL, High-density lipoprotein; TC, Total cholesterol; LDL, Low-density lipoprotein; NT, not tested; TG, Triacylglycerols; WBC, white blood cells
Table 3. Patient characteristics according to nutritional status (SGA + s-albumin) in KTX group

| Parameters                      | Total Sample Median (% or IQR or SD) | Not at Risk Median (IQR) | At Risk for Malnutrition Median (IQR) |
|--------------------------------|-------------------------------------|--------------------------|--------------------------------------|
| Subjects                       | 128                                 | 92 (71.9%)               | 36 (28.1%)                           |
| Sex                            |                                     |                          |                                      |
| Male                           | 72 (56.3%)                          | 51 (55.4%)               | 21 (58.3%)                           |
| Female                         | 56 (43.7%)                          | 41 (44.6%)               | 15 (41.7%)                           |
| BMI                            | 3–7                                 | 6–7                      | ≤5                                   |
| FAT (%)                        | 25.87±4.12                          | 26.26±4.24               | 24.89±3.68                           |
| FTO                            | 34.20±8.98                          | 34.32±9.16               | 33.85±8.57                           |
| LTM (%)                        | 12.40±4.55                          | 12.61±4.70               | 11.76±4.07                           |
| LTI                            | 50.55±12.04                         | 50.81±12.00              | 49.77±12.38                          |
| TBW (%)                        | 12.68±2.50                          | 13.01±2.53*              | 11.70±2.20*                          |
| ECW (L)                        | 48.92±6.34                          | 48.67±6.18               | 49.64±6.81                           |
| IOW (L)                        | 17.71±3.62                          | 17.84±3.68               | 17.32±3.45                           |
| E/I (L)                        | 0.97±0.14                           | 0.94±0.14*               | 1.04±0.13*                           |
| OH (%)                         | 11.83±9.38                          | 10.41±9.87*              | 15.88±6.41*                          |
| Arm circumference (cm)         | 30.43±3.70                          | 31.34±3.58*              | 28.02±2.88*                          |
| Serum albumin level (g/L)      | 35.40±6.21                          | 36.37±6.68               | 33.00±3.28                           |
| Age (years)                    | 51.00±13.65                         | 48.47±13.18*             | 57.44±12.83*                         |
| Time after TX (months)         | 39.17±6.18                          | 39.75±6.25               | 37.62±5.96                           |
| ADMA (µM/L)                    | 0.96±0.31                           | 0.95±0.32                | 0.97±0.30                            |
| FGF-23 (pg/ml)                 | 570.14±840.86                       | 515.33±800.50            | 688.06±923.62                        |
| hs-CRP (mg/L)                  | 4.85±4.10                           | 4.67±4.13                | 5.26±4.04                            |
| Diabetes                       | 41 (31.0%)                          | 24 (26.1%)               | 17 (42.2%)                           |
| CVD                            | 42 (31.8%)                          | 25 (27.2%)               | 17 (47.2%)                           |

* p<0.05; ADMA, Asymmetric dimethylarginine; FGF-23, Fibroblast growth factor-23; IL-6, Interleukine-6; SGA, Subjective Global Assessment; BMI, Body Mass Index; CRP, C-reactive protein; hs-CRP, High sensitivity C-reactive protein; FTI, Fat tissue index; LTM, Lean tissue mass; LTI, Lean tissue index; TBW, Total body water; ECW, Extracellular water; IOW, Intracellular water; E/I, Extracellular water/Intracellular water ratio; OH, Overhydration

RESULTS

Anthropometry

Body mass index correlated positively with body fat (r=0.54, p<0.05), arm (r=0.79, p<0.05) and waist (r=0.84, p<0.05) circumferences respectively. The mean percentage of body fat was 34.20±8.98% (range, 4.3–55.6%). Fat tissue index (FTI) was 12.40±4.55 kg/m², and lean tissue index (LTI) was 12.68±2.50 kg/m². Patients with excessive body mass were identified by BMI; 35.4% of KTRs were classified as overweight and 20.8% were obese.

In our study group, KTRs with altered body composition – excessive body mass (BMI ≥25) with the simultaneous decline of lean tissue index < 12 (LTI, 11.56±1.37 kg/m²) and high fat tissue index >14 (FTI, 16.59±3.89 kg/m²) – presented a significant and approximately two-fold increase in the level of hs-CRP (6.51±4.68 mg/L vs. 3.14±3.14 mg/L), than patients with normal range fat tissue index <14). The nutritional status and anthropometric characteristics of kidney transplant recipients are included in Table 3.

Cardiovascular risk factors

Cardiovascular diseases (CVDs) were present in 31.8% of KTRs, and the prevalence of CVD in relation to BMI is presented in Table 4. Inflammatory and endothelial dysfunction markers in the study and control group did not differ significantly. Only the FGF 23 level was significantly higher in KTRs (p<0.05).

The negative correlation between ADMA and serum albumin (r=−0.41, p<0.05) and positive with overhydration (OH%, r=0.28, p<0.05) was noticed. Moreover, administration of statins and/or angiotensin-converting enzyme inhibitors were significantly related to lower ADMA levels in KTRs (no statins 1.0±0.31 vs. statins 0.79±0.24; no ACEI 0.99±0.3 vs. ACEI 0.75±0.3 µM/L, p<0.05).

We also identified a significant positive correlation between FGF-23 levels and inflammatory markers (hs-CRP, IL-6) and negative with an overall index of re
nal function (eGFR-CKD EPI). Detailed information on inflammatory and endothelial dysfunction parameters in study groups is included in Table 5 and Table 6. The correlations between studied parameters are presented in Table 7 and Figs 1–3.

Multivariate regression

Multivariate regression model was established to predict the impact of the independent variables, related to nutritional status (SGA, s-albumin), on the prevalence of CVD in the kidney transplant recipients’ group (the adjusted R2 of the model was 0.36; \( p < 0.002 \)). Analysis showed that nutritional status assessed by SGA and s-albumin may have a significant impact on the prevalence of CVD in the kidney transplant recipients’ group.

Detailed information is provided in Table 8.
Therefore, precise effects of ADMA excess – involving abnormalities of the endothelial cell function, angiogenesis process, vascular injury repair, NO and ROS altered generation, in conjunction with the severity of CKD, profoundly affect cardiovascular morbidity and mortality (Liu et al., 2018). According to Zoccali’s et al. (2001) prospective evaluation of 225 hemodialysis patients, an increase in plasma concentration of asymmetrical dimethylarginine was the second (after age) strong risk factor of overall mortality and cardiovascular mortality in the ESRD population. Moreover, according to Fliser and others study (Fliser et al., 2005), the elevated ADMA level may contribute to significantly faster (52.8 mo vs. 71.6 mo) nephropathy progression. In our study group of KTRs, plasma ADMA concentration correlated negatively with the eGFR ratio, emphasizing its importance in the process of renal impairment. According to Kielstein and others (Kielstein et al., 2004), elevated levels of ADMA cause decreased renal perfusion. Possible mechanisms of diminished renal plasma flow might be related to proteinuria, which leads to the damage of renal tubules cells, subsequently, DDAH enzyme insufficient renal expression, caused by tubular dysfunction, results in the excess of ADMA concentration in the blood and tissues (Kielstein et al., 2007; Raptis et al., 2013). As a consequence, renal perfusion continually decreases, with its negative effects on ADMA cumulative concentration, kidney impairment progression and hypertension. In our study group statistical analysis confirmed a significant positive correlation between ADMA concentration and proteinuria ($r=0.35$, $p<0.05$); additionally, we also identified a negative correlation with serum albumin ($r=-0.42$, $p<0.05$).

Elevated inflammatory markers (CRP, IL-6), together with ADMA high plasma levels in subjects with advanced renal failure, according to numerous studies (Zoccali et al., 2001; Zoccali et al., 2002; Aucella et al., 2009; Tripepi et al., 2011), remain in clinically confirmed correlation and play an important role as a predictive factor for atherosclerotic changes, cardiovascular events and overall mortality.

Based on the results obtained from the current study, the correlation between ADMA and serum albumin ($r=-0.41$, $p<0.05$) and overhydration (OH%, $r=0.28$, $p<0.05$) are strongly suggested. Also, regression analysis shows an association between the nutritional status (albumin) and CVD which may suggest that low albumin as an acute phase protein and indicator of malnutrition is associated with a higher risk of endothelium damage. According to Frenay and others (Frenay et al., 2015), certain kidney transplant recipient subpopulation classified with ADMA concentration $\geq 0.66$ $\mu$mol/l was characterized by excessive albuminuria and also elevated levels of FGF-23, PTH and serum phosphorus concentration.

According to our findings, ADMA elevated plasma concentration in the subgroup of patients with the presence of CVDs were non-statistically significant, however, we have observed an upward trend in comparison to KTRs without CVDs and in the control group, nonetheless, comparison to healthy subjects is still recommended. The suppressive effect of hyperglycemia on DDAH enzyme activity, due to diabetes mellitus and/or insulin resistance, may partly explain a presumptive mechanism of ADMA excessive accumulation in these conditions, however, a comprehensive pharmacotherapy approach to
the treatment of diabetes mellitus (with metformin and thiazolidinediones usage) might have a positive impact on ADMA regulation (Napora et al., 2006).

Numerous studies have suggested the positive therapeutic impact of statin administration on circulating asymmetric dimethylarginine reduction. According to Serban’s and others (Serban’s et al., 2015) meta-analysis study, the hydrophic statin administration (rosuvastatin, pravastatin and fluvastatin), but not hydrophobic statins, significantly decreased plasma ADMA concentration. In the present study, the administration of statins and/or angiotensin-converting-enzyme inhibitors was significantly related to a lower level of ADMA in KTRs.

FGF-23

Fibroblast growth factor 23 (FGF-23), as a regulatory component of renal phosphate reabsorption and 1,25(OH)2D synthesis, is a crucial part of mineral and bone homeostasis (Chu et al., 2021). Laboratory evaluation of FGF-23 concentration in humans, allows the detection of two available types of FGF-23 – an intact FGF-23 form (iFGF-23) and C-terminal fibroblast growth factor 23 form (cFGF-23), additionally, the cFGF-23 ELISA assay might be able to provide more precise results (Chu et al., 2021), since, according to recent findings (Sharma et al., 2020; Chu et al., 2021), inflammation and iron status might interfere with the effects of iFGF-23 correlations. Notwithstanding those reservations, in our study, we evaluated the plasma concentration of intact fibroblast growth factor 23 form in KTRs and control subjects.

Current literature strongly suggest that an increased circulating level of FGF-23 is independently associated with cardiovascular and all-cause mortality after kidney transplantation (Wolf et al., 2011; Baia et al., 2013), overall graft loss in kidney transplant recipients (Wolf et al., 2011; Chu et al., 2021) and progression of chronic kidney disease (CKD) (Wolf et al., 2011). Moreover, recent research emphasizes plasma FGF-23 importance in the process of inflammation, erythropoiesis, functional iron deficiency and anaemia (David et al., 2016; David et al., 2017; Edmonston et al., 2020; Mace et al., 2020). Our findings suggest the link between considerably diminished renal function evaluated by reduced glomerular filtration rate and the higher fibroblast growth factor 23 concentration in KTRs.

Additionally, a significant correlation for FGF-23, obtained in our study group, has been associated with hs-CRP (r=0.46, p<0.05), IL-6 (r=0.4, p≤0.05), hemoglobin (r=−0.55, p<0.05) and ferritin (r=0.56, p<0.05).

Research results submitted by Malyszko et al. (2014), which concern the mechanisms of endothelial cell injury in kidney transplant recipients, have also emphasized the importance of disturbances in the FGF23-Klotho system for future cardiac outcomes (Liebeuf et al., 2018). According to Yilmaz and others (Yilmaz et al., 2013), donor type (living or deceased) (Baia et al., 2014), time after transplantation (Malyszko et al., 2014), mean corpuscular volume (Malyszko et al., 2014) and more. Our study findings clearly suggest a statistically significant positive correlation between FGF-23 levels and inflammatory markers (hs-CRP, IL-6) and negative with graft function (eGFR-CKD EPI, eGFR-MDRD).

LIMITATIONS AND OUTLOOK

Our findings suggest that the inflammatory and endothelial dysfunction markers, as well as nutritional status, are considerably altered in kidney transplant recipients. The recent interest in novel endothelial dysfunction markers creates an opportunity for early prediction of future cardiac outcomes and/or renal disease progression. Accurate diagnosis and early treatment are crucial for improving patient outcomes. Further research is needed to entirely understand the link between investigated biomarkers and their association with life-threatening complications of the cardiovascular system. The limitation of our study is the relatively small group of patients, but despite its limitation, the results of the present study are valuable because they indicate a problem of occurrence of untraditional risk factors of CVD also in patients after TX, such as inflammation and nutritional status. Further studies in KTRs (e.g., multicenter) are needed to confirm our results.

CONCLUSIONS

Nutritional status, inflammation and endothelial dysfunction markers (ADMA, FGF-23) are considerably altered even in stable kidney transplant recipients. hs CRP, IL-6 and ADMA were comparable to CKD patients, and FGF 23 was higher in KTRs. The upward trend of elevated ADMA plasma concentration in KTRs with the presence of CVDs as well as the link between hs-CRP and the higher blood level of fibroblast growth factor 23 – were observed in our study. The presented study indicated a problem of occurrence of untraditional risk factors of CVD in patients after kidney transplantation, such as inflammation and deterioration of the nutritional status.

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