BANTAO Journal 2023; 21(1): 10-16

BJ

BANTAO Journal

Original article

Clinical and Laboratory Markers for Diabetic Nephropathy in Patients with Type 2 Diabetes Mellitus

Stanislava Ilieva[1], Emilia Naseva[2] and Lachezar Lozanov[1]

[1]Clinic of Internal Diseases, Acibadem CityClinic University Hospital Tokuda, [2]Faculty of Public Health "Prof. Tsekomir Vodenicharov, MD, DSc", Medical University of Sofia, Sofia, Bulgaria

Abstract

Introduction. Diabetic nephropathy (DN) is a leading cause of ESRD worldwide and an independent risk factor for cardio-vascular mortality in diabetic patients. The multifactorial pathogenesis of DN is illustrated by many scientific researches. Nevertheless, there are no affirmed biomarkers of its presence, except albuminuria and kidney biopsy still stays the only method that confirms the diagnosis. The aim of our study is to reveal a relationship between certain biomarkers and the development of DN in patients with type 2 Diabetes Mellitus (DM).

Methods. Eighty-one patients are studied (49 males and 32 females), age 22 to 75 years. All of them are with CKD and histologically proven nephropathies, regardless of the kidney function. Forty-eight of subjects are with T2DM and the rest (33) are without DM. The patients are divided into 3 groups, according to histological findings: 1-st group (n=30): patients with DM and DN; 2-nd group (n=18): diabetic patients with other nephropathies but non DN and 3-rd group: patients without DM. Kidney function, lipid profile, IL-6, CRP, fibrinogen, D-dimer, homocysteine, folic acid, methylenetetrahydrofolate reductase gene polymerphism (MTHFR A1289C and C677T) are tested in the three groups.

Results. Serum homocysteine is significantly increased in patients with DN (p=0,034), compared to diabetic patients without DN. Also, there is a significantly increased level of serum IL-6 (p=0,019) and serum fibrinogen (p=0,012) in all diabetic patients, compared to non-diabetic patients. There is no significant difference between the rest of biomarkers among the three groups.

Conclusion. No single biomarker can be a predictor of DN, but a combination of biomarkers should be searched in larger studies.

Keywords: biomarkers, chronic kidney disease, diabetes mellitus type 2, diabetic nephropathy, predictors

Introduction

Microvascular complications of Diabetes mellitus (DM) are of major health and social importance. Diabetic nephropathy (DN) is a leading cause of end-stage renal disease (ESRD) worldwide and also an independent risk factor of cardio-vascular mortality in diabetic patients [1]. Approximately 90% of all diabetic patients are with type 2 DM. The rest 10% are with type 1 DM and some other rare types, as MODY (Maturity-onset diabetes of the young) and secondary types in pancreatitis, Cushing syndrome, and corticosteroid treatment [2,3]. It is found that nearly 50% of all patients with type 2 DM have also a chronic kidney disease (CKD), which is more common in certain populations as elderly patients, obese, some ethnic groups, low social and economic status [4]. The problem is not only the large number of diabetic patients, but also the late diagnosis in an advanced stage with minimal or no therapeutic options. The early diagnostics of CKD in patients with type 2 DM includes clinical and laboratory tests, as well as a histological verification with percutaneous puncture kidney biopsy [5]. The latter shows the presence of DN and other non-diabetic nephropathies (primary glomerulonephritis, autoimmune diseases and etc.) which is crucial for the treatment. Percutaneous puncture kidney biopsy is an invasive procedure and is not applicable in all patients. The restrictions of its performance are associated with the presence of advanced parenchymal changes, small kidneys, renal asymmetry, comorbidity, uncontrolled hypertension, severe anemia, concomitant anticoagulant and antiplatelet medication [6]. This provoke investigators to search for non-invasive methods with a high predictive value of presence of diabetic kidney injury and its prognosis.

The aim of our study was to reveal a relationship between certain clinical and laboratory biomarkers which are routine for the clinical practice and development of DN in patients with type 2 DM.

Correspondence to: Stanislava Ilieva, Clinic of Internal Diseases, Acibadem CityClinic University Hospital Tokuda, Sofia, Bulgaria; E-mail: stanislava_ilieva@hotmail.com

Material and methods

The study enrolled 81 biopsied patients between the age of 22 and 75 years with histologically proven nephropathies, hospitalized at Nephrology Department, Clinic of Internal Diseases, Acibadem CityClinic University Hospital Tokuda in Sofia, Bulgaria from 2018-2023. Forty-nine of them were male (60,5%) and 32(39,5%) - female. Forty-eight of all patients (59,3%) were with type 2 DM and the rest 33(40,7%) were without diabetes. Patients were divided into 3 groups according to the presence of DM and DN in renal histology: I group: Patients with type 2 DM and DN (n=30); male 22(73,3%) and female 8(26,7%). II group: Patients with type 2 DM without DN in the biopsy (n=18); male 11(61,1%) and female 7(38,9%). III group: Non-diabetic patients (n=33); male 16(48,5%); female 17 (51,5%).

Indications for performing renal biopsy were: a presence of albuminuria or proteinuria of different range or impaired renal function.

Patients were assessed for having arterial hypertension and diabetic complications: diabetic retinopathy, polyneuropathy, macroangiopathy (for the I and II group). The presence of diabetic retinopathy was confirmed after fundoscopy by ophthalmologist. Diabetic neuropathy was accepted on the basis of neurological consultation and in some cases- electromyography test. Diabetic macroangiopathy involved cases with a history of ischemic heart disease or peripheral artery disease.

In all 3 groups the following parameters were measured: serum creatinine and e-GFR (CKD-EPI), cystatin C, BMI, lipid status (Low density lipoproteins-LDL, High density lipoproteins-HDL, Triglycerides), uric acid, glycated hemoglobin, inflammatory markers (CRP, IL-6), indexes of blood clotting and vessel damage (fibrinogen, D-dimer), homocysteine, folic acid, genetic factors (methylenetetrahydrofolate reductase gene polymorphism MTHFR A1289C and MTHFR C677T) as well as the thyroid function (TSH, FT4, Antithyroid microsomal antibody - MAT, Antithyroglobulin antibody - TAT). All laboratory tests were performed at the Clinical Laboratory of Acibadem CityClinic University Hospital Tokuda, Sofia.

From the statistical point of view the results are presented as number and proportion of patients in each group as well as the mean± standard deviation for normally distributed variables. The shape of the distribution was assessed by Kolmogorov-Smirnov and Shapiro-Wilk tests. Pearson Chi-Square test (Fisher Exact test when applicable) was performed to check the relationship of categorical variables.

Results

The mean age of patients in the 3 groups was as follows: group (diabetic patients with DN) - 62±8; II group (diabetic patients without DN) - 61±9; III group (non-diabetic patients) 46±14.

There was no significant difference regarding duration of DM between I and II group. In the I group the duration was 13±8 years and in the II group - 12±5 years, respectively (p=0,505).

Patients from the 3 groups were at different stages of CKD. Distribution of patients according to the stages of CKD (KDIGO Classification) is presented in Table 1.

Table 1. Distribution of patients according to the stages of CKD (KDIGO Classification)

	I group	II group	III group
CKD 1	n=6(20.0%)	n=2(11.1%)	n=10(30.3%)
CKD 2	n=3(10.0%)	n=7(38.9%)	n=4(12.1%)
CKD 3a	n=4(13.3%)	n=1(5.6%)	n=5(15.2%)
CKD 3b	n=0(0.0%)	n=0(0.0%)	n=0(0.0%)
CKD 4	n=12(40.0%)	n=8(44.4%)	n=13(39.4%)
CKD 5	n=5(16.7%)	n=0(0.0%)	n=1(3.0%)

Data presented as number of patients and percent.
Abbreviation: CKD – Chronic kidney disease.

Histological findings in the 3 groups are as follows:

In the I group, despite DN, 3(10.0%) of patients have hypertensive nephropathy; 5(16.7%) have tubulointerstitial changes; 1(3.3%) has FSGS; 1(3.3%) has membranous nephropathy; 1(3.3%) has C3- Glomerulonephritis. So, 11(36.6%) out of 30 patients with DN have another co-existing non-diabetic disease.

In the II group of diabetic patients with CKD, renal histology didn`t show DN, but we found the following diagnosis: predominant number of patients-10(55.5%) have a combination of hypertensive vascular changes and tubulointerstitial lesions; 3(16.6%) of patients have only hypertensive nephropathy and 1(5.5%) patient has tubulointerstitial nephritis alone; 2(11.1%) have membranous nephropathy; 1(5.6%)-membranoproliferative nephropathy; 1(5.6%)-mesangioproliferative glomerulonephritis with deposition of C3.

In the III group non-diabetic patients, we found: 7(21.2%) patients have chronic tubulointerstitial nephritis, 7(21.2%) have FSGS; 6(18.2%) have IgA nephropathy; 4(12.1%) have lupus nephrites; 3(9.1%) have membranous nephropathy; 3(9.1%) have membranoproliferative glomerulonephritis; 2(6.1%) have idiopathic nephrotic syndrome and 2(6.1%) - other.

Regarding diabetic complication, the comparison between the two diabetic groups revealed a significant difference in the presence of diabetic retinopathy. Fifty percent of patients with DN have a kind of retinopathy, whereas no one from diabetic patients without DN has it. There was no significant difference between the two groups in respect of other diabetic complications (Table 2).

Table 2. Comparison of diabetic complications between groups I and II

		I group-DM with DN	II group-DM without DN	P for groups 1 and 2
Diabetic retinopathy	Yes	n=15(50.0%)	n=0(0.0%)	<0.001
	No	n=15(50.0%)	n=18(100.0%)	
Diabetic polyneuropathy	Yes	n=20(66.7%)	n=10(55.6%)	0.441
	No	n=10(33.3%)	n=8(44.4%)	
Diabetic macroangiopathy	Yes	n=10(33.3%)	n=7(38.9%)	0.697
	No	n=20(66.7%)	n=11(61.1%)	
Diabetic gangrene	Yes	n=2(6.7%)	n=0(0.0%)	0.263
	No	n=28(93.3%)	n=18(100.0%)	

Data presented as number of patients and percent.
Abbreviation: DM - Diabetes Mellitus, DN – Diabetic Nephropathy.

Table 3. Comparison of clinical parameters between the 3 groups

		I group-DM with DN	II group-DM without DN	III group-Non-diabetics	P for groups 1 and 2	P for groups 1 and 3	P for groups 2 and 3	P for groups (1+2) and 3
BMI	18.0-24.9	n=1 (3.3%)	n=1 (5.6%)	n=11 (33.3%)	0.747	<0.001	0.013	<0.001
	25.0-29.0	n=6 (20.0%)	n=5 (27.8%)	n=13 (39.4%)				
	>30.0	n=23 (76.7%)	n=12 (66.7%)	n=9 (27.3%)				
Arterial hypertension	Yes	n=30 (100.0%)	n=18 (100.0%)	n=28 (84.8%)		0.054	0.148	0.009
	No	n=0 (0.0%)	n=0 (0.0%)	n=5 (15.0%)				

Data presented as number of patients and percent.
Abbreviation: DM - Diabetes Mellitus, DN – Diabetic Nephropathy, BMI – Body Mass Index.

Table 4. Comparison of metabolic parameters between the 3 groups

		I group – DM with DN	II group DM without DN	III group non-diabetics	P for groups 1 and 2	P for groups 1 and 3	P for groups 2 and 3	P for groups (1+2) and 3
LDL	Low	n=4 (14.3%)	n=5 (27.8%)	n=1 (3.3%)	0.307	0.322	0.024	0.092
	Normal	n=22 (78.6%)	n=13 (72.2%)	n=26 (86.7%)				
	High	n=2 (7.1%)	n=0 (0.0%)	n=3 (10.0%)				
HDL	Low	n=19 (67.9%)	n=11 (61.1%)	n=9 (30.0%)	0.639	0.013	0.093	0.007
	Normal	n=9 (32.1%)	n=7 (38.9%)	n=20 (66.7%)				
	High	n=0 (0.0%)	n=0 (0.0%)	n=1 (3.3%)				
Triglycerides	Normal	n=15 (50.0%)	n=10 (55.6%)	n=24 (72.7%)	0.709	0.064	0.214	0.062
	High	n=15 (50.0%)	n=8 (44.4%)	n=9 (27.3%)				
Uric acid	Normal	n=24 (80.0%)	n=11 (61.1%)	n=28 (61.1%)	0.154	0.613	0.085	0.204
	High	n=6 (20.0%)	n=7 (38.9%)	n=5 (15.2%)				
HbA1C	Normal	n=14 (46.7%)	n=9 (50.0%)		0.823			
	>6.5%	n=16 (53.3%)	n=9 (50.0%)					

Data presented as number of patients and percent.
Abbreviation: DM - Diabetes Mellitus, DN – Diabetic Nephropathy.

Other clinical features that we compared between the 3 groups are the presence of obesity and arterial hypertension. The results are presented in the table 3.
There was a significant prevalence of obesity (BMI >30.0) in diabetic patients, regardless the presence of DN, compared to non-diabetic patients. Everybody with diabetes in our study has arterial hypertension and most patients without DM have arterial hypertension as well.

Table 5. Comparison of inflammatory and coagulation factors between the 3 groups

		I group DM with DN	II group DM without DN	III group Non-diabetics	P for groups 1 and 2	P for groups 1 and 3	P for groups 2 and 3	P for groups (1+2) and 3
CRP	Normal	n=17 (56.7%)	n=13 (72.2%)	n=26 (78.8%)	0.281	0.060	0.732	0.119
	High	n=13 (43.3%)	n=5 (27.8%)	n=7 (21.2%)				
IL-6	Normal	n=17 (56.7%)	n=14 (77.8%)	n=29 (87.9%)	0.139	0.005	0.430	0.019
	High	n=13 (43.3%)	n=4 (22.2%)	n=4 (12.1%)				
D-dimer	Normal	n=13 (43.3%)	n=10 (55.6%)	n=22 (66.7%)	0.412	0.063	0.433	0.095
	High	n=17 (56.7%)	n=8 (44.4%)	n=11 (33.3%)				
	Low	n=1 (3.3%)	n=0 (0.0%)	n=0 (0.0%)				
Fibrinogen	Normal	n=14 (46.7%)	n=11 (61.1%)	n=27 (81.8%)	0.508	0.012	0.177	0.021
	High	n=15 (50.0%)	n=7 (38.9%)	n=6 (18.2%)				

Data presented as number of patients and percent.
Abbreviation: DM - Diabetes Mellitus, DN – Diabetic Nephropathy.

Table 6. Comparison of homocysteine, folic acid and thyroid function between the 3 groups

		I group DM with DN	II group DM without DN	III group non-diabetics	P for groups 1 and 2	P for groups 1 and 3	P for groups 2 and 3	P for groups (1+2) and 3
Homocysteine	Normal	n=9 (30.0%)	n=11 (61.1%)	n=11 (33.3%)	0.034	0.777	0.056	0.448
	High	n=21 (70.0%)	n=7 (38.9%)	n=22 (66.7%)				
Folic acid	Normal	n=20 (87.0%)	n=13 (100.0%)	n=27 (81.8%)	0.323	0.338	0.219	0.242
	High	n=3 (13.0%)	n=0 (0.0%)	n=6 (18.2%)				
	Low	n=1 (3.3%)	n=0 (0.0%)	n=3 (9.1%)				
TSH	Normal	n=29 (96.7%)	n=16 (88.9%)	n=27 (81.8%)	0.135	0.140	0.416	0.220
	High	n=0 (0.0%)	n=2 (11.1%)	n=3 (9.1%)				
	Low	n=2 (6.9%)	n=0 (0.0%)	n=1 (3.0%)				
FT4	Normal	n=27 (93.1%)	n=17 (94.4%)	n=30 (90.9%)	0.240	0.326	0.753	0.640
	High	n=0 (0.0%)	n=1 (5.6%)	n=2 (6.1%)				
TAT Ab	Normal	n=26 (86.7%)	n=15 (83.3%)	n=29 (87.9%)	0.999	0.885	0.686	0.751
	High	n=4 (13.3%)	n=3 (16.7%)	n=4 (12.1%)				
MAT Ab	Normal	n=29 (96.7%)	n=15 (83.3%)	n=26 (78.8%)	0.142	0.033	0.999	0.096
	High	n=1 (3.3%)	n=3 (16.7%)	n=7 (21.2%)				

Data presented as number of patients and percent.
Abbreviation: DM - Diabetes Mellitus, DN – Diabetic Nephropathy.

With respect of the metabolic status, we didn`t find any significant difference between diabetic patients with DN and those without DN. We only found that HDL is significantly lower in patients with DN compared to non-diabetic patients (p=0.013). Also, it is significantly lower in two diabetic groups, as compared to the non-diabetic patients (p=0.007). Glycemic control didn`t show any difference between diabetic patients with DN and those without DN. Glycated hemoglobin (HbA1C) was above 7.5% in almost half of the patients from the two groups (Table 4).

We compared the presence of some inflammatory markers (CRP, IL-6) and coagulation factors (fibrinogen, D-dimers) between the 3 groups. We didn`t find any significant difference between diabetic patients with and without DN, but there were a significantly higher levels of IL-6 in diabetic patients with nephropathy compared to non-diabetic patients (p 0.005). In addition, levels of fibrinogen appeared to be higher in diabetic patients with nephropathy than in non-diabetic patients (p 0.012) (Table 5).

We found that the levels of homocysteine are signifycantly elevated in diabetic patients with DN compared to DM patients without DN (p 0.034), although there was no significant difference between patients with DM and those without DM. There was no difference in the levels of folic acid and thyroid function between the 3 groups (Table 6).

In our study we investigated the carriage of mutations of methylenetetrahydrofolate reductase gene (MTHFR A1289C and C677T) as genetic factors for development of diabetic nephropathy. We found out that the carriage of pathological alleles is widespread and it doesn`t play any role for the manifestation of DN (Table 7).

Table 7. Carriage of pathological alleles of methylenetetrahydrofolate gene (MTHFR A1289C and C677T) among the 3 groups

		I group DM with DN	II group DM without DN	III group non-diabetics	P for groups 1 and 2	P for groups 1 and 3	P for groups 2 and 3	P for groups (1+2) and 3
MTHFR A1289C	Homozygous AA normal	n=16 (53.5%)	n=10 (55.6%)	n=15 (45.5%)	0.982	0.794	0.785	0.721
	Heterozygous	n=12 (40.0%)	n=7 (38.9%)	n=16 (48.5%)				
	Homozygous CC mutation	n=2 (6.7%)	n=1 (5.6%)	n=2 (6.1%)				
MTHFR C677T	Homozygous AA normal	n=13 (43.3%)	n=6 (33.3%)	n=7 (21.2%)	0.770	0.094	0.300	0.084
	Heterozygous	n=12 (40.0%)	n=8 (44.4%)	n=22 (66.7%)				
	Homozygous CC mutation	n=5 (8.7%)	n=4 (22,2%)	n=4 (12.1%)				

Data presented as number of patients and percent.
Abbreviation: DM - Diabetes Mellitus, DN – Diabetic Nephropathy.

Discussion

Diagnosing etiology of CKD in patients with DM is a challenge for the clinicians. Kidney biopsy is seldom considered, except in cases with atypical presentation as: lack of diabetic retinopathy, short duration of diabetes (under 5 years), presence of micro- or macrohematuria, active urinary sediment, sudden onset of gross proteinuria or nephrotic syndrome, acute kidney injury, suspicion of an autoimmune disease and markers of hepatitis B or C [7]. As in many trials in the literature, in our study approximately 1/3 of diabetic patients (37.5%) have non-diabetic kidney injury, 1/3 (36.6%) have a combination of diabetic and non-diabetic lesions and the rest 39.5% - diabetic nephropathy alone [8].

In our study, the group of diabetic patients without DN showed a similar duration of DM as those with DN and suggests that the predictive value of duration is not a strong one.

Diabetic retinopathy (DR) is present significantly in 50% of patients with DN, whereas no one in the II group has it. So, the existence of DR might be of a high predictive value for the presence of DN. It has been confirmed by large studies [9,10]. Severity of DR could be a marker of progression of CKD [11]. If DR is established, a screening for Diabetic Kidney Disease (DKD) should be performed regularly.

In our study, diabetic polyneuropathy (DPN) is found in most of the patients with DN (66.7%) and in more than half of diabetic patients without DN (55.6%). It indicates a high prevalence of polyneuropathy among diabetic patients with CKD and its predictive value should be an object of further research. In the literature there are publications, stating a relationship between DPN and DKD [12,13].

As it is observed in our study, the predominant number of patients from the 3 groups are at CKD 4, which might indicate a progressive course of the kidney disease.

Regarding histological findings it is worth mentioning that a significant number of patients with DN (36.6%) have another co-existing non-diabetic injury. At the same time, most of the patients from the II group

(77.7%) have hypertensive and/or tubulointerstitial lesions. That states the question if these vascular or tubulointerstitial changes could be associated with DM and/or if they could be a manifestation of DKD. The role of tubulointerstitial damage in DKD has been recently researched by many authors [14-16]. That explains the fact that many diabetic patients reach ESRD without a significant albuminuria [17-19].

Obesity and arterial hypertension presented significantly in all diabetic patients in our study, compared to non-diabetic patients. Although they don`t show an existence of DN, they could deteriorate the course of CKD in patients with DM [20].

We studied the role of IL-6 as a marker of DN and found that it was significantly higher in patients with DN compared to the non-diabetic patients, although there was no significant difference between the two diabetic groups. IL-6 is an inflammatory cytokine with a pleiotropic action. It is involved in the mechanisms of obesity and insulin resistance [21,22]. The mechanism of its action is impairing phosphorylation of the insulin receptor and inhibition of insulin signaling to the cells [21]. Also, IL-6 unlocks molecular mechanisms (gp-130-STAT 3 dependent mechanisms) that lead to an adaptive immune response, cellular infiltration and inflammatory process [23]. Several studies in literature demonstrate the role of IL-6 for developing DN [24,25]. Shikano *et al*. found a significant elevation of serum IL-6 in patients with microalbuminuria and overt proteinuria, compared to normoalbuminuric patients, as well as a significant correlation with fibrinogen [26].

Fibrinogen is a 340kD plasma protein, containing two sets of α-, β- and γ-chains. At the beginning of coagulation process thrombin cuts off fibrin-peptides from the N-end of α and β chains, which leads to polymerrization of fibrin monomers in insoluble fibrin set [27]. Mechanisms of microvascular injury are due to changes of blood viscosity, activation of thrombogenesis and erythrocyte aggregation in the terrain of impaired endothelial function and vascular reactivity [28]. Several studies in the literature demonstrate elevated levels of fibrinogen in patients with DM and DN [29-31]. In our trial, levels of IL-6 and fibrinogen were significantly higher in diabetic patients, compared to non-diabetic patients. It is not sufficient to accept the role of these biomarkers as single predictors of DN, but they could also be indicators of renal injury in diabetic patients.

We investigated the role of homocysteine, folic acid and methylenetetrahydrofolate reductase gene polymerphism (MTHFR A1289C and C677T) in patients from the 3 groups. Methylenetetrahydrofolate reductase (MTHFR) is an enzyme, which takes part in the process of remethylation of homocysteine to methionine. Inadequate enzyme activity causes elevation of homocysteine serum levels. MTHFR is encoded by MHTFR -gene, which is located in humans in chromosome 1, locus p36.3 [32]. Different sequences in the DNA molecule determine so called genetic polymorphism, which has 24 genetic variants [33]. The most investigated ones are C677T and A1289C. In 1998 Neugebauer *et al*. established the role of MTHFR gene polymorphism as a risk factor for DN in DM type 2 [34]. In our study, we found that carriage of pathological alleles 677T and 1289C is widespread among the studied population and it couldn`t be used as a biomarker of DN.

Interestingly, in our trial homocysteine appeared to be significantly higher in patients with DN, than diabetic patients without DN, although there was no significant difference between diabetic and non-diabetic patients. Homocysteine is a sulfur containing amino acid, which is formed as a result of intracellular metabolism of methionine [35]. Its unfavorable action in the kidneys and vascular system is associated with an endothelial dysfunction, increased formation of reactive oxygen species (ROS), activation of vasoconstrictor and depression of vasodilator substances, increased extracellular matrix deposition. The relationship between elevated plasma concentration of homocysteine and the level of albumin excretion in diabetics has been described in some trials [36,37]. Wang *et al*. demonstrated a positive correlation between homocysteine, albumin excretion and the degree of reduction of e-GFR for a 4 years period of follow-up [38]. Some authors consider homocysteinemia as an independent predictor of kidney injury in early stages of kidney disease [38,39]. The small number of patients in our study does not allow us to accept homocysteine as a single biomarker of DN, but its high levels might predict a progressive course of renal disease.

Conclusion

Multifactorial pathogenesis of DN reveals different metabolic disorders in diabetic patients with CKD. That`s why it is so difficult to determine a particular biomarker, except albuminuria, as a hallmark of DN. None of the studied biomarkers in our trial could be a single predictor of DN and a combination of biomarkers should be searched in larger studies. Since renal biopsy stays the only method that determines diagnosis and prognosis, we recommend to perform it in each patient with elevated albumin or protein excretion or impaired renal function, if there are not contraindications for it.

Conflict of interest statement. None declared.

References

1. Collins AJ, Kasiske B, Herzog C, *et al*. United States Renal Data System 2006. Annual Data Report. *American Journal of Kidney Diseases* 2007; 49: S1-S234.
2. Winer N, Sowers JR. Epidemiology of Diabetes. *J Clin Pharmacol* 2004; 44: 397-405.

3. Forouhi N, Wareham NJ. Epidemiology of Diabetes. *Medicine* 2014; 12: 698-702.
4. Thomas M, Cooper M, Zimmet P. Changing epidemiology of type 2 diabetes mellitus and associated chronic kidney disease. *Nat Rev Nephrol* 2016; 12: 73-81.
5. Persson F, Rossing P. Diagnosis of diabetic kidney disease: state of the art and future perspective. *Kidney Int* Suppl (2011), 2018; 8(1): 2-7.
6. Schnuelle P. Renal Biopsy for Diagnosis in Kidney Disease: Indication, Technique and Safety. *J Clin Med* 2023; 12(19): 6424.
7. Ritz E. Clinical manifestation and natural history of diabetic kidney disease. *Med Clin N Am* 2013; 96: 19-29.
8. Espinel E, Agraz I, *et al*. Renal Biopsy in Type 2 Diabetic Patients. *J Clin Med* 2015; 4(5): 998-100.
9. Jeng CJ, Hsieh YT, Yang CM, *et al*. Diabetic Retinopathy in Patients with Diabetic Nephropathy: Development and Progression. *PLoS ONE* 2016; 11(8): e0161897.
10. He F, Xia X, Wu XF, *et al*. Diabetic retinopathy in predicting nephropathy in patients with type 2 diabetes and renal disease: a metaanalysis. *Diabetologia* 2013; 56: 457-466.
11. Saini CD, Kochar A, Poonia R. Clinical correlation of diabetic retinopathy with nephropathy and neuropathy. *Indian J Ophtalmol* 2021; 69(11): 3364-3368.
12. Nabrdalik K, Kwiendacz H, Moos J, *et al*. Diabetic Peripheral Neuropathy is associated with Diabetic Kidney Disease and Cardiovascular Disease: The Silesia Diabetes-Heart Project. *Current Problems in Cardiol* 2023; 48(8): 101726.
13. Yang Z, Lou X, Zhang J, *et al*. Association Between Early Markers of Renal Injury and Type 2 Diabetic Peripheral Neuropathy. *Diabetes Metab Syndr Obes* 2021; 14: 4391-4397.
14. Lizuka K, Deguchi K. Diabetic renal tubulointerstitial disease. *Front Endocrinol* 2023; 14: 1303514.
15. Lim BJ, Yang JW, Zou J, *et al*. Tubulointerstitial fibrosis can sensitize the kidney to subsequent glomerular injury. *Kidney Int* 2017; 92(6): 1395-1403.
16. Zeni L, Norden AGW, Cancarini G, Unwin RJ. A more subtelocentric view of diabetic kidney disease. *J Nephrol* 2017; 30(6): 701-717.
17. Afroz T, Sagar R, Reddy S, Rajaram KG. Clinical and histological correlation of diabetic nephropathy. *Saudi Journal of Kidney Diseases and Transplantation* 2017; 28(4): 836-841.
18. Halimi JM. The emerging concept of chronic kidney disease without clinical proteinuria in diabetic patients. *Diabetes & Metabolism* 2012; 38(4): 291-297.
19. Budhiraja P, Thajudeen B, Popovtzer M. Absence of albuminuria in type 2 diabetics with classical diabetic nephropathy: clinical pathological study. *Journal of Biomedical Science and Engineering* 2013; 6: 20-25.
20. Maric C, Hall JE. Obesity, metabolic syndrome and diabetic nephropathy. *Contrib Nephrol* 2011; 170: 28-35.
21. Kristiansen O, Mandrup-Poulsen T. Interleukin-6 and Diabetes. The Good, the Bad or the Indifferent. *Diabetes* 2005; 54(suppl 2): S114-S124.
22. Rehman K, Akash M, *et al*. Role of Interleukin-6 in Development of Insulin Resistance and Type 2 Diabetes Mellitus. *Crit Rev Eukaryot Gene Expr* 2017; 27(3): 229-236.
23. Feigerlova E, Battaglia-Hsu SF. IL-6 signaling in diabetic nephropathy: From pathophysiology to therapeutic perspectives. *Cytokine and Growth Factor Reviews* 2017; 37: 57-65.
24. Shikano M, Sobajima H, Yoshikawa H, *et al*. Usefulness of a Highly Sensitive Urinary and Serum IL-6 Assay in Patients with Diabetic Nephropathy. *Nephron* 2000; 85: 81-85.
25. Chondary N, Ahlawal RS. Interleukin-6 and C-Reactive Protein in Pathogenesis of Diabetic Nephropathy. New Evidence Linking Inflammation, Glycemic Control and Microalbuminuria. *Iranian Journal of Kidney Diseases* 2008; 2(2): 72-79.
26. Lord ST. Molecular mechanisms affecting fibrin structure and stability. *Arterioscler Thromb Vasc Biol* 2011; 31: 494-499.
27. Lominadze D, Dean WL, Tyagi SC, Roberts AM. Mechanisms of fibrinogen-induced microvascular dysfunction during cardiovascular disease. *Acta Physiol (Oxf)* 2010; 198: 1-13.
28. Kaur S, Singh P, Indu V, Singla G. Fibrinogen, Lp(a), microalbuminuria and left ventricular mass index: cardiovascular disease factors in diabetes. *Indian J Clin Biochem* 2012; 27: 94-96.
29. Mohan G, Kaur R, Aggarwal A, Singh P. To study levels of serum fibrinogen in type 2 diabetes mellitus and its association with diabetic microvascular complications. *Int J Adv Med* 2017; 4: 10-14.
30. Sun J, Lin C. Correlation of vascular endothelial function and coagulation factors with renal function and inflammatory factors in patients with diabetic nephropathy. *Exp Ther Med* 2018; 16: 4167-4171.
31. Blom HJ, Smulders Y. Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects. *J Inherit Metab Dis* 2011; 34(1): 75-81.
32. Goyett P, Sumner JS, Milos R, *et al*. Human methylentetrahydrofolate reductase: isolation of cDNA mapping and mutation identification. *Nat Genet* 1994; 7(4): 551.
33. Sibani S, Christensen B, O`Ferrall E, *et al*. Characterization of six novel mutations in the methylentetrahydrofolate reductase (MTHFR) gene in patients with homocystinuria. *Human Mutation* 2000; 15(3): 280-287.
34. Neugebauer S, Baba T, Watanabe T. Methylenetetrahydrofolate reductase gene polymorphism as a risk factor for diabetic nephropathy in NIDDM patients. Lancet 1998: 352(9126): 454.
35. Selhub J. Homocysteine metabolism. *Annu Rev Nutr* 1999; 19: 217-246.
36. Chico A, Perez A, O'Ferrall E, *et al*. Plasma homocysteine is related to albumin excretion rate in patients with diabetes mellitus: a new link between diabetic nephropathy and cardiovascular disease? *Diabetologia* 1998; 41: 684- 693.
37. Mao S, Xiang W, Huang S, Zhang A. Association between homocysteine status and the risk of nephropathy in type 2 diabetes mellitus. *Clinica Chimica Acta* 2014; 431(20): 206-210.
38. Wang H, Cui K, Xu K , Xu S. Association between plasma homocysteine and progression of early nephropathy in type 2 diabetic patients. *Int J Clin Exp Med* 2015; 8(7): 11174-11180.
39. Vaidya VS, Waikar SS, Ferguson MA, *et al*. Urinary biomarkers for sensitive and specific detection of acute kidney injury in humans. *Clin Transl Sci* 2008; 1: 200-208.

BANTAO Journal 2023; 21(1): 17-22

BJ

BANTAO Journal

Original article

Use of Eplerenone in Kidney Transplant Recipients with Heart Failure: Case Series

Nikolina Basic-Jukic, Slavica Potočki, Ivana Juric, Lea Katalinic, Vesna Furic-Cunko, Zoran Sabljić, Armin Atic, Marina Kljajic and Bojan Jelakovic

Department of nephrology, arterial hypertension, dialysis and transplantation, Clinical hospital centre Zagreb and School of medicine, university of Zagreb, Zagreb, Croatia

Abstract

Introduction. Despite their potential benefits, mineralocorticoid receptor antagonists are rarely used in kidney transplant recipients due to the fear of complications. We aim to describe epidemiological, clinical and laboratory characteristics, treatment, and outcomes of kidney transplant patients treated with eplerenone.
Methods. Kidney transplant recipients who received eplerenone were included in this single-center retrospective study. Serum electrolytes, uric acid, estimated glomerular filtration rate (eGFR), and proteinuria were recorded.
Results. Ten kidney transplant recipients (6 male) received eplerenone for treatment of heart failure. With the median follow-up of 16 months (range 6-78 months), nine were alive with the functioning kidney allograft. Eplerenone was well tolerated. Only one patient developed mildly elevated potassium and one developed severe symptomatic hyponatremia, requiring hospitalization. The glomerular filtration rate decreased after eplerenone's introduction from 47.5(42-54) to 40 (35-48) ml/min/1.73m^2 at three months ($p<0.01$) and remained stable in follow-up. Proteinuria decreased from 303 mg/24h to 185 mg/24h without a statistically significant difference. Eplerenone did not influence cyclosporine or tacrolimus trough levels. Uric acid increased after the introduction of eplerenone, requiring increased doses of allopurinol.
Conclusions. Eplerenone may be used with caution in kidney transplant recipients. Careful monitoring of all electrolytes and not only of potassium is mandatory. In our cohort, eplerenone increased uric acid levels. Further studies are required to elucidate the clinical benefits and safety of eplerenone in this patient population.

Key words: eplerenone, heart failure, kidney transplant, hyperuricemia, hyponatremia, mineralocorticoid receptor antagonist

Introduction

Cardiovascular diseases are the leading cause of death, accounting for up to 60% of all lethal outcomes after kidney transplantation [1]. All major phenotypes of cardiovascular diseases are present in kidney transplant recipients, including heart failure, coronary artery disease, valvular heart disease, peripheral vascular disease, cerebrovascular diseases, arrhythmias, and pulmonary hypertension. The shared risk factors between kidney failure and cardiovascular disease, such as diabetes and hypertension, can partially explain this phenomenon. However, end-stage kidney disease (ESKD) can additionally exacerbate cardiac problems due to anemia, fluid overload, uremic toxins, and secondary hyperparathyroidism with vascular calcifications that contribute to the development and exacerbation of atherosclerosis, coronary artery disease, and left ventricular hypertrophy. Finally, mineralocorticoid excess, which is present in ESKD, significantly contributes to the risk of cardiac disease. Mineralocorticoid receptor (MR) activation is involved in inflammation, fibrosis, and progression of chronic kidney disease. Besides the kidneys, MR is expressed in many other organs, including the colon, heart, central nervous system, and brown adipose tissue, which are responsive to aldosterone signaling. Aldosterone contributes to blood pressure control and maintains extracellular volume homeostasis by provoking renal sodium reabsorption and potassium excretion [2].
Eplerenone is a second-generation steroidal mineralocorticoid receptor antagonist (MRA) that selectively binds to the MR, blocking aldosterone binding and inhibiting sodium reabsorption and other aldosterone-mediated mechanisms. It is more selective in the MRA effect and devoid of androgen antagonism and progesterone agonism than spironolactone. Additionally, the absence of its long-acting metabolites could be associated with less frequent adverse events. However, the use of eplerenone still carries a risk of hyperkalemia [3]. Thus, despite the potential benefits, MRAs are ra-

Correspondence to: Nikolina Basic-Jukic, Department of nephrology, arterial hypertension, dialysis and transplantation, Clinical hospital centre Zagreb, Kispaticeva 12, 10000 Zagreb, Croatia; E-mail: nina_basic@net.hr; nbasic@kbc-zagreb.hr

rely used in kidney transplant recipients (KTR) due to the fear of complications. Herein, we report our experience with the use of eplerenone after kidney transplantation.

Material and methods

We conducted a retrospective, observational cohort study that included patients who underwent kidney transplantation at the University Hospital Centre Zagreb, Croatia, and received eplerenone therapy. The patient's electronic medical records were used to extract the relevant data. Data extracted from the patients' database included their age, gender, primary kidney disease, dialysis vintage, posttransplant complications, and laboratory data before the introduction of eplerenone, three months after the start of treatment, and at the last follow-up. Laboratory measurements included estimated glomerular filtration rate (eGFR), uric acid, potassium, sodium, and 24-h proteinuria. Patients were advised to limit their potassium-rich food intake. The research was conducted in accordance with the Declaration of Helsinki. The University Hospital Center Zagreb Ethics Committee reviewed and approved this study protocol.

The primary endpoint was the tolerance to eplerenone, assessed by the occurrence of the adverse events: occurrence of hyperkalemia (>5.1 mmol/L), decrease in eGFR >30% from baseline, or any adverse event that required discontinuation of eplerenone. Efficacy outcomes of the present analysis included a CV composite outcome of nonfatal myocardial infarction, stroke, or hospitalization for heart failure. Changes in proteinuria, uric acid, and electrolytes from baseline to the end of the study were also analyzed.

Categorical data were presented by absolute and relative frequencies. The Shapiro-Wilk test tested the normality of the continuous variable distribution. The median and the interquartile range described continuous data. Logistic regression analysis was used to analyze the independent factors associated with decreased eGFR and increased serum uric acid. The level of significance was set at an Alpha of 0.05. Considering the relatively small sample size and the possibility of overfitting in the multivariate logistic regression model, we adopted a forward stepwise method (probability for stepwise: entry $p<0.05$, removal $p>0.1$) for logistic regression analysis to reduce the number of independent variables entering the model. There was no substitution for the missing data. The statistical analysis was performed using MedCalc® Statistical Software version 20.023 (MedCalc Software Ltd., Ostend, Belgium; https://www.medcalc.org; 2021) and the IBM SPSS Stat. 23 (IBM Corp. Released 2015., Ver. 23.0. IBM Corp; Armonk, NY, USA).

Results

Out of 2226 KTRs who underwent kidney transplantation at our institution, ten patients were treated with eplerenone. There were 6 male and 4 female patients, with a median age of 75 (range 61 to 84). The immunosuppressive protocol was based on calcineurin inhibitors (tacrolimus in 2, cyclosporine in 5 patients) or mTORi (everolimus in 3 patients). Eight patients received mycophenolate.

All patients had arterial hypertension, four had a history of myocardial infarction, 3 had a stroke, seven had peripheral arterial disease, and seven had cardiac arrhythmia. Their characteristics are presented in Table 1.

Table 1. Characteristics of patients treated with eplerenone

	n=10
Age (years)	75(61-84)
Gender ratio (M/F)	6/4
Dialysis vintage (years)	3(1-8)
Time from transplantation (years)	13(8-27)
Primary kidney disease, n / Total	
Diabetic nephropathy	1/10
Glomerulonephritis	2/10
ADPKD	3/10
Nefroangiosclerosis	1/10
Endemic nephropathy	2/10
Analgetic nephropathy	1/10
CNI, n / Total	
Tacrolimus	2/7
Cyclosporine	5/7
Mycophenolate, n / Total	8/10
mTORi, n / Total	3/10
Steroid dose (n = 9)	5(2.5-10)
Myocardial infarction, n / Total	4/10
Stroke(n = 3) , n /Total	
1 episode	2/3
2 episodes	1/3
Peripheral vascular disease, n / Total	7/10
Arrhythmia, n / Total	7/10
Acute rejection, n / Total	1/10
CMV reactivation, n / Total	1/10
Malignant tumor, n / Total	5/10
Hypertension, n / Total	10/10
Number of antihypertensive drugs, n/Total	
2	2/10
3	3/10
4	2/10
5	3/10
Posttransplant diabetes, n / Total	2/10
Heart echo, n / Total	
EF (%)	65(22-65)
Pulmonary artery pressure	45(40-90)
Valvular disease, n / Total	3/10
NTproBNP 0	5539(2235-118070)
NTproBNP final	2740(1500-4596)

Data are expressed by median (range)

At the last outpatient visit, with the median follow-up of 16 months (range 6-78 months), nine patients were alive with functioning kidney allograft. One of them died from heart failure 26 months after the introduction

of eplerenone.

The glomerular filtration rate decreased from 47.5 (IQR, 42-54) ml/min/1.73m^2 after eplerenone's introduction to 40.0 (IQR, 35-48) ml/min/1.73m^2 and remained stable in follow-up. Proteinuria decreased from 303 mg/24h to 185 mg/24h without a statistically significant difference (Table 2).

Table 2. Estimated glomerular filtration rate significantly decreased after introduction of eplerenone. Uric acid levels increased during the treatment, not reaching the statistical significance (p=0.06). Data are represented as median and range. *p<0.05 vs. baseline

	Median (IQR)			*P**
	At baseline	**3 months**	**Last follow-up**	
Body weight	80(67-88)	81.5(68-88)	76.5(69-89)	0.91
eGFR	47.5(42-54)	40.0(35-48)	39.0(33-48)	**0.01**†
SBP	138(128-167)	132(125-158)	137(122-160)	0.72
DBP	87(76-108)	82(74-105)	84(72-103)	0.58
Proteinuria	303(219-378)	241(128-263)	185(118-407)	0.64
Potassium	4.3(4.1-4.7)	4.3(4.0-4.5)	4.1(3.8-4.6)	0.76
Sodium	140.5(139-141)	141(137-142)	140(139-142)	0.97
Calcium	2.35(2.32-2.43)	2.43(2.36-2.47)	2.43(2.34-2.51)	0.28
Uric acid	380(364-450)	442(389-521)	430(421-471)	0.06

IQR-interquartil range, *Friedman's Test, †at the level of P<0,05 significantly higher values at baseline compared to other measurements

Only one patient developed mild hyperkaliemia (serum potassium increased from 4.9 to 5.2 mmol/L). Other patients had potassium levels within the normal range, including those with mildly elevated potassium at the baseline. Uric acid increased in all but one patient, requiring either the introduction or increase of the existing dose of allopurinol or febuxostat.

Calcium levels were stable after the introduction of eplerenone.

Two patients required hospitalizations after the introduction of eplerenone. One patient developed severe hyponatremia one month after the start of treatment with 25 mg eplerenone. She was admitted to the hospital with mental deterioration and a serum sodium level of 113 mmol/L. On physical examination, she was dysponoic, with a heart rate of 99 bpm, blood pressure of 136/69 mmHg, with edema and hyperemia of the lower extremities. Heart auscultation revealed an ejection systolic murmur without radiation to the carotids. The electrocardiogram (ECG) showed atrial fibrillation with a normal ventricular response. A chest radiograph showed an increased cardiothoracic index with cranial vascular redistribution-the transthoracic echocardiogram calcification of the mitral valve, with moderate MV regurgitation. There was also a severe tricuspid valve (TV) regurgitation, secondary to TV annulus dilation. The left ventricular ejection fraction (LVEF) was preserved (60%), and the left ventricle was not dilated. Eplerenone was immediately omitted, and hyponatremia was conservatively treated. The patient recovered within five days and was discharged with stable kidney allograft function. The other patient was hospitalized due to the heart failure. He died with a functioning kidney allograft.

There were no significant changes in blood pressure during the follow-up, and we did not perform continuous ambulatory blood pressure monitoring. All patients required at least two antihypertensive drugs to control arterial hypertension.

Tacrolimus and cyclosporine trough levels were stable, without significant oscillations after the introduction of eplerenone. There was no need to change the dose of everolimus after the introduction of eplerenone.

Discussion

This study showed an acceptable safety profile of eplerenone in KTR with heart failure. Nine out of ten patients were alive at the last outpatient visit. Eplerenone was associated with a decline in eGFR at three months, which remained stable after that, and increased uric acid levels in follow-up. A decline in proteinuria was recorded but did not reach statistical significance. One patient developed severe symptomatic hyponatremia, requiring hospitalization. Only one patient developed mild hyperkaliemia.

Cardiovascular diseases are the leading cause of death in patients with ESKD. Despite detailed cardiac evaluation in kidney transplant candidates, different cardiovascular complications, including heart failure, remain the most common cause of death after kidney transplantation [4]. All other cardiovascular conditions, including coronary artery disease, valvular heart disease, peripheral vascular disease, cerebrovascular diseases, arrhythmias, and pulmonary hypertension, significantly affect posttransplant outcomes. Additionally, kidney transplantation is increasingly accessible to elderly recipients with a more pronounced burden of different comorbidities, including diabetes, coronary heart disease, or heart failure. All these conditions have been associated with the upregulation of mineralocorticoid receptors [5]. Mineralocorticoid receptor upregulation results with increased transcription of profibrotic genes, including TGF-beta1, plasminogen acti-

vator inhibitor 1, connective tissue growth factor, and extracellular matrix proteins, which are linked to renal and cardiac fibrosis, adding to the risk of cardiovascular disease [2]. In different clinical settings, mineralocorticoid receptor antagonists (MRA) may have antihypertensive and antiproteinuric effects. However, they also prevent ischemia-reperfusion injury, the transition from acute, chronic injury to chronic kidney disease (CKD), the progression of CKD, and the prevention of cardiovascular outcomes [6]. Mineralocorticoid receptor knockout in smooth muscle cells, but not in the endothelium, prevented cyclosporine-induced nephrotoxicity [7]. In experimental models, MR blockade efficiently ameliorated calcineurin toxicity, possibly by preventing increased renal vascular resistance in acute CIN [8]. The majority of these conditions already develop in kidney transplant recipients before transplantation or evolve during the posttransplant follow-up period.

For this reason, MRAs may represent an essential therapeutic option in kidney transplant patients [9], hypothetically protecting transplanted organs from different injuries. Mineralocorticoid receptor antagonists were found to be effective in patients with resistant hypertension [10], heart failure, or myocardial infarction [11,12], presenting an attractive and additive treatment after kidney transplantation. However, despite the obvious multiple potential benefits of MRA in this particular group of patients, which carries a heavy burden of cardiovascular problems, they are rarely prescribed due to the fear of complications.

Hyperkaliemia is most frequently considered a problem after the introduction of MRAs. A recent clinical trial investigated the safety of eplerenone 25 mg/day in 31 kidney-transplanted patients receiving cyclosporine. Patients with serum potassium levels ≥5 mmol/L or a history of severe hyperkalemia (≥6 mmol/L) were excluded from the trial, whereas 61% of patients were treated with angiotensin convertase enzyme inhibitor or angiotensin receptor blocker. Eight patients experienced mild hyperkalemia (>5 mmol/L) while treated with eplerenone, and one had moderate hyperkalemia (>5.5 mmol/L) and received potassium-exchange resin. One patient developed acute kidney allograft failure that was attributed to diarrhea [13]. In our cohort, only one patient developed mild hyperkaliemia. It is important to stress that all patients from our cohort were educated about the risk of hyperkaliemia before introducing eplerenone. They were advised to avoid potassium-rich food.

Serum sodium changes frequently complicate cardiovascular diseases. Sodium balance is fine-tuned in the distal parts of the nephron, where eplerenone exhibits some of its pleiotropic effects. Out of a total of 6632 patients with myocardial infarction and heart failure included in the EPHESUS trial randomized to either eplerenone or placebo, 6221 had a post-baseline sodium measurement. Seven hundred ninety-seven patients developed hyponatremia, and 1476 developed hypernatremia. Patients treated with eplerenone had lower mean serum sodium over the follow-up (140 vs. 141 mmol/L; $p<0.0001$) and more often developed hyponatremia episodes (15 vs. 11% $p=0.0001$) and less often hypernatremia episodes (22 vs. 26% $p=0.0003$) [14). In our study, only one patient developed hyponatremia, but a severe form with neurological presentation requiring hospitalization. This case indicates that sodium should be regularly checked along with other electrolytes after the introduction of eplerenone.

Hyperuricaemia is a common problem after kidney transplantation. Diuretics are one of the most important and frequent causes of secondary hyperuricemia that may either increase uric acid reabsorption and/or decrease uric acid secretion. Serum uric acid was found to be an independent predictor of all-cause and cardiovascular mortality but also acute coronary syndrome, stroke, and heart failure [15]. However, the threshold levels of serum uric acid that can contribute to cardiovascular risk significantly are not defined [16].

The association of spironolactone with increased serum uric acid levels is controversial. Previous results suggested that spironolactone does not increase serum uric acid levels [17]. Later, Cabrera et al. showed that low-dose spironolactone increases serum uric acid levels in patients with chronic kidney disease [18]. In Ohta *et al*.'s study, eplerenone treatment increased serum uric acid levels while indapamide decreased them [19]. In our group, uric acid increased from the median 380 to 430 umol/L, not reaching the statistical significance ($p=0.06$), probably due to the small sample size.

In our study, eplerenone decreased proteinuria over the follow-up; however, it was without statistical significance. Proteinuria is a specific problem in the kidney transplant population associated with numerous etiologic factors. Early use of MRA may prevent kidney allograft deterioration in patients with proteinuria and non-immunological lesions on kidney biopsies. Additionally, it may have a beneficial effect on other forms of proteinuria. However, this hypothesis needs to be evaluated.

The use of eplerenone did not significantly alter our patients' blood pressure. However, we did not have continuous ambulatory blood pressure monitoring, so we may have missed the beneficial effect of eplerenone on 24-hour blood pressure control.

Solid organ recipients are usually not included in randomized clinical studies due to the potential risk of interactions with immunosuppressive medications. Eplerenone does not inhibit or induce CYP3A4, which results in a neutral effect on calcineurin inhibitor levels. There is also no drug–drug interaction between mycophenolic acid and eplerenone [13].

Current data suggest that finerenone, a novel, more selective MRA, protects against kidney disease progre-

ssion and cardiovascular events in patients with type 2 diabetes and chronic kidney disease [20]. Finerenone has been shown to reduce the urinary albumin-to-creatinine ratio in patients with chronic kidney disease receiving a renin-angiotensin antagonist but with less pronounced effects on serum potassium levels than spironolactone [21-25]. Other non-steroidal MRAs (esaxerenone and apararenone) have also been shown to significantly reduce albuminuria in CKD patients in phase 2 clinical trials [26]. In the recently published European Renal Association (ERA) synopsis for nephrology practice of the 2023 European Society of Hypertension (ESH) Guidelines for the Management of Arterial Hypertension, MRAs have not been commented as a potential treatment for KTR [27].

The current data add to the body of information regarding the safety of eplerenone in the kidney transplant population. However, the present study had certain limitations, which should be mentioned. It is a retrospective and observational study, providing associations rather than causation. Given the small sample size and cardiac indication for using eplerenone, we could not precisely evaluate the potential renoprotective effect. Selecting patients from a single transplant center could reduce our findings' generalizability.

In conclusion, eplerenone may be used cautiously in kidney transplant recipients with eGFR>35 ml/m2/1.73m2. Careful monitoring of all electrolytes and not only of potassium is mandatory. In our cohort, eplerenone increased uric acid levels. Further studies are required to elucidate eplerenone's and other MRA's clinical benefits and safety in this patient population.

Conflict of interest statement. None declared.

Reference

1. Awan AA, Niu J, Pan JS, *et al*. Trends in the Causes of Death among Kidney Transplant Recipients in the United States (1996–2014). *Am J Nephrol* 2018; 48: 472-481.
2. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, *et al*. Chronic kidney disease and cardiovascular risk: Epidemiology, mechanisms, and prevention. *Lancet* 2013; 382: 339-352.
3. Gomez-Sanchez EP. Third-generation mineralocorticoid receptor antagonists: why do we need a fourth? *J Cardiovasc Pharmacol* 2016; 67(1): 26-38.
4. Kes P, Brunetta B, Basic-Jukic N. Cardiovascular diseases after kidney transplantation. *Lijec Vjesn* 2006; 128(7-8): 228-232.
5. Mende CW, Samarakoon R, Higgins PJ. Mineralocorticoid Receptor-Associated Mechanisms in Diabetic Kidney Disease and Clinical Significance of Mineralocorticoid Receptor Antagonists. *Am J Nephrol* 2023; 54(1-2): 50-61.
6. Barrera-Chimal J, Girerd S, Jaisser F. Mineralocorticoid receptor antagonists and kidney diseases: pathophysiological basis. *Kidney Int* 2019; 96(2): 302-319.
7. Amador CA, Bertocchio JP, Andre-Gregoire G, *et al*. Deletion of mineralocorticoid receptors in smooth muscle cells blunts renal vascular resistance following acute cyclosporine administration. *Kidney Int* 2016; 89(2): 354-362.
8. Sun QL, Li M, Rui HL, Chen YP. Inhibition of local aldosterone by eplerenone reduces renal structural damage in a novel model of chronic cyclosporine A nephrotoxicity. *J Renin Angiotensin Aldosterone Syst* 2015; 16(2): 301-310.
9. Bramlage P, Swift SL, Thoenes M, *et al*. Non-steroidal mineralocorticoid receptor antagonism for the treatment of CV and renal disease. *Eur J Heart Fail* 2016; 18: 28-37.
10. Williams B, MacDonald TM, Morant S, *et al*. Spironolactone versus placebo, bisoprolol, and doxazosin to determine the optimal treatment for drug-resistant hypertension (PATHWAY-2): a randomised, double-blind, crossover trial. *Lancet* 2015; 386: 2059-2068.
11. Zannad F, Gattis Stough W, Rossignol P, *et al*. Mineralocorticoid receptor antagonists for heart failure with reduced ejection fraction: integrating evidence into clinical practice. *Eur Heart J* 2012; 33: 2782-2795.
12. Girerd N, Ferreira JP, Rossignol P, *et al*. A tentative interpretation of the TOPCAT trial based on randomized evidence from the brain natriuretic peptide stratum analysis. *Eur J Heart Fail* 2016; 18: 1411-1414.
13. Bertocchio JP, Barbe C, Lavaud S, *et al*. Safety of Eplerenone for Kidney-Transplant Recipients with Impaired Renal Function and Receiving Cyclosporine A. *PLoS One* 2016; 11(4): e0153635.
14. Martens P, Ferreira JP, Vincent J, *et al*. Serum sodium and eplerenone use in patients with a myocardial infarction and left ventricular dysfunction or heart failure: insights from the EPHESUS trial. *Clin Res Cardiol* 2022; 111(4): 380-392.
15. Maloberti A, Mengozzi A, Russo E, *et al*. The Results of the URRAH (Uric Acid Right for Heart Health) Project: A Focus on Hyperuricemia in Relation to Cardiovascular and Kidney Disease and its Role in Metabolic Dysregulation. *High Blood Press Cardiovasc Prev* 2023; 30(5): 411-425.
16. Kuwabara M, Kodama T, Ae R, *et al*. Update in uric acid, hypertension, and cardiovascular diseases. *Hypertens Res* 2023; 46(7): 1714-1726.
17. Falch DK, Schreiner A. The effect of spironolactone on lipid, glucose and uric acid levels in blood during long-term administration to hypertensives. *Acta Med Scand* 1983; 213: 27-30.
18. Cabrera SE, Edwards NC, Steeds RP, *et al*. Spironolactone increases serum uric acid levels in patients with chronic kidney disease. *J Hum Hypertens* 2014; 28: 2101.
19. Ohta Y, Ishizuka A, Hayashi S, *et al*. Effects of a selective aldosterone blocker and thiazide-type diuretic on blood pressure and organ damage in hypertensive patients. *Clin Exp Hypertens* 2015; 37(7): 569-573.
20. Ruilope LM, Pitt B, Anker SD, *et al*. Kidney outcomes with finerenone: an analysis from the FIGARO-DKD study. *Nephrol Dial Transplant* 2023; 38(2): 372-383.
21. Bakris GL, Agarwal R, Chan JC, *et al*. Effect of finerenone on albuminuria in patients with diabetic nephropathy: a randomized clinical trial. *JAMA* 2015; 314: 884-894.
22. Pitt B, Kober L, Ponikowski P, *et al*. Safety and tolerability of the novel non-steroidal mineralocorticoid receptor antagonist BAY 94-8862 in patients with chronic heart failure and mild or moderate chronic kidney disease: a randomized, double-blind trial. *Eur Heart J* 2013; 34: 2453-2463.
23. Bakris GL, Agarwal R, Anker SD, *et al*. Effect of finerenone on chronic kidney disease outcomes in type 2 diabetes. *N Engl J Med* 2020; 383: 2219-2229.
24. Pitt B, Filippatos G, Agarwal R, *et al*. Cardiovascular events with finerenone in kidney disease and type 2 diabetes. *N Engl J Med* 2021; 385: 2252-2263.

25. Ruilope LM, Pitt B, Anker SD, *et al*. Kidney outcomes with finerenone: an analysis from the FIGARO-DKD study. *Nephrol Dial Transplant* 2023; 38(2): 372-383.
26. Alexandrou M-E, Theodorakopoulou MP, Sarafidis PA. Role of Mineralocorticoid Receptor Antagonists in Diabetic Kidney Disease. *Kidney and Dialysis* 2022; 2: 163-182.
27. Sarafidis P, Schmieder R, Burnier M, *et al*. A European Renal Association (ERA) synopsis for nephrology practice of the 2023 European Society of Hypertension (ESH) Guidelines for the Management of Arterial Hypertension. *Nephrol Dial Transplant* 2024; 39(6):929-943.