ORIGINAL ARTICLE

Renalase, kidney function, and markers of endothelial dysfunction in renal transplant recipients

Edyta Zbroch, Jolanta Małyszko, Jacek Małyszko, Ewa Koc-Żórawska, Michał Myśliwiec

Department of Nephrology and Transplantology, Medical University of Białystok, Białystok, Poland

KEY WORDS

blood pressure, kidney function, kidney transplantation, renalase

ABSTRACT

INTRODUCTION Renalase is an enzyme released by the kidneys, which breaks down catecholamines in the blood and thus may regulate blood pressure. In kidney transplant recipients, endothelial dysfunction is often present.

OBJECTIVES The aim of the study was to assess associations between renalase, blood pressure, and kidney function in kidney allograft recipients.

PATIENTS AND METHODS We studied 62 kidney allograft recipients. Complete blood count, urea and creatinine levels, serum lipids, and fasting glucose were measured by standard laboratory methods. We also assessed markers of coagulation: prothrombin fragments 1+2; fibrinolysis: tissue plasminogen activator (tPA), plasminogen activator inhibitor, plasmin-antiplasmin complexes; endothelial function/injury: von Willebrand factor (vWF), thrombomodulin, intercellular adhesion molecule, vascular cell adhesion molecule (VCAM); and inflammation: high-sensitivity C-reactive protein and interleukin 6. Renalase levels were assessed using a commercially available kit.

RESULTS Mean serum renalase levels in kidney allograft recipients correlated with age, time after transplantation, soluble CD44 (sCD44), VCAM, serum creatinine, estimated glomerular filtration rate (eGFR; measured by CKD-EPI, MDRD, and Cockcroft-Gault formulas), serum phosphate, urea, sCD146, vWF, and thrombomodulin and tended to correlate with tPA. In patients with eGFR above 60 ml/min, renalase was lower than in those with lower eGFR. In hypertensive allograft recipients, renalase was significantly higher than in normotensives. A multiple regression analysis showed that renalase was predicted in 58% by serum creatinine.

CONCLUSIONS Renalase, which is highly elevated in kidney transplant recipients, is dependent primarily on kidney function, which deteriorates with age and time after transplantation. Further studies are needed to establish the putative role of renalase in the pathogenesis of hypertension after transplantation and its possible use in novel targeted therapies.

Correspondence to:
Edyta Zbroch, MD, PhD, Klinika Nefrologii i Transplantologii, Uniwersytet Medyczny w Białymstoku, ul. Żurawia 14, 15-540 Bialystok, Poland, phone: +48-85-740-94-64, fax: +48-85-743-45-86, e-mail: edzbroch@poczta.onet.pl
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INTRODUCTION The endothelial cell layer is the "guardian" of molecular traffic between the blood and surrounding tissue, and endothelial integrity plays a pivotal role in many aspects of vascular function, e.g., control of vasomotor tone and permeability. Cardiovascular risk factors such as hypertension may cause endothelial dysfunction and even disintegration, finally resulting in small vessel disappearance (vascular rarefaction) and tissue hypoxia. In patients with chronic kidney diseases (CKD), ongoing endothelial damage in the capillary system of the renal medulla and accompanying vascular rarefaction are thought to be central processes toward progressive kidney damage.[1,2] Endothelial dysfunction is highly prevalent in both cardiovascular disease and CKD.[3-5]

Among various functions of the kidney, it is also an endocrine organ. In 2005, Xu et al.[6] presented in their landmark study the history of

TABLE Clinical and biochemical characteristics of kidney transplant recipients

age, y	45.8 ±312.18
time after transplantation, mo	43.62 ±40.05
hemoglobin, g/l	135.2 ±15.2
erythrocyte count, × 10^{12}/l	4.22 ±0.62
leukocyte count, × 10^{9}/l	7.12 ±2.02
creatinine, µmol/l	137.9 ±53.04
eGFR by MDRD, ml/min	56.01 ±20.04
eGFR by CKD-EPI, ml/min	57.82 ±21.66
creatinine clearance by Cockcroft-Gault, ml/min	55.31 ±19.07
IL-6, pg/ml	0.50 (0.04–5.48)
hsCRP, mg/l	1.16 (0.02–12.76)
ICAM, ng/ml	256 ±89
VCAM, ng/ml	626 ±482
thrombomodulin, ng/ml	7.36 ±5.74
vWF, %	171 ±29
tPA, ng/ml	9.00 ±8.74
PAI-1, ng/ml	38.12 ±28.10
F1+2, nmol/l	6.94 ±4.23
TAT, µg/l	7.21 ±5.57
PAP, µg/l	651 ±546
sCD44, ng/ml	754 ±202
sCD146, ng/ml	277 ±112
sCD40L, ng/ml	1.00 (0.5–14.0)
SBP, mmHg	135 ±11
DBP, mmHg	84 ±7
renalase, µg/ml	6.72 ±2.86

Data are presented as mean ± standard deviation or median and ranges.

Abbreviations: CKD-EPI – Chronic Kidney Disease Epidemiology Collaboration, DBP – diastolic blood pressure, eGFR – estimated glomerular filtration rate, hsCRP – high-sensitivity C-reactive protein, F1+2 – prothrombin fragments 1+2, ICAM – intercellular adhesion molecule, IL-6 – interleukin 6, MDRD – Modification of Diet in Renal Disease, PAI-1 – plasminogen activator inhibitor-1, PAP – plasmin-antiplasmin complexes, SBP – systolic blood pressure, TAT – thrombin-antithrombin complexes, tPA – tissue plasminogen activator, VCAM – vascular cell adhesion molecule, vWF – von Willebrand factor

the discovery of renalase and its possible role in hypertension. Xu et al.[6] reported that renalase had a flavin-adenine dinucleotide (FAD)-binding domain, and that FAD was an essential cofactor for the stability and its monoamine oxidase activity. Renalase is preferentially expressed in proximal tubules, but also in the glomeruli and distant tubules, cardiomyocytes, liver, and skeletal muscles[6] as well as in peripheral nerves, adrenals, endothelium, central nervous system, a 12.5-day-old rat embryo, and human adipose tissue.[6,7] It degrades catecholamines in vitro with a distinct substrate specificity and inhibitor profile, indicating that it represents a new class of flavin adenine dinucleotide-containing monoamine oxidases. It lowers blood pressure in vivo by decreasing cardiac contractility and heart rate and by preventing the expected compensatory increase in peripheral vascular tone.[8]

Due to the fact that the endothelium is multifunctional and highly distributed among all organ systems, its dysfunction is not restricted anatomically to the heart, nor is it limited in disease scope to atherosclerosis. Considering the fact that endothelial dysfunction is present in kidney allograft recipients[9] and renalase is expressed in the endothelium, the aim of the present study was to assess associations between renalase, and markers of endothelial dysfunction in kidney allograft recipients.

PATIENTS AND METHODS The study included 62 kidney allograft recipients (47 men). Before transplantation, all kidney transplant recipients were on renal replacement therapy. The immunosuppressive regimen of kidney transplant recipients consisted of calcineurin inhibitor, cyclosporine (mean levels, 162.60 ±52.71 ng/ml), in combination with mycophenolate mofetil (2 g/d) and prednisone (median, 7.5 mg/d; ranges, 0–10 mg/d). They also received antihypertensive drugs, including calcium channel blockers (100%), β-blockers (59%), angiotensin-converting inhibitors (24%), diuretics (26%), together with statins (21%).

All patients maintained sufficient and stable graft function, showed no clinical signs of rejection, or inflammation. All subjects gave written informed consent, and the protocol was approved by the Medical University Ethics Committee.

Blood was drawn in the morning when patients presented for routine control visits after an overnight fast. Glomerular filtration rate (GFR) was estimated using the simplified Modification of Diet in Renal Disease formula (MDRD; estimated GFR [eGFR] = 186.3 × serum creatinine [mg/dl]$^{-1.14}$ × age$^{-0.203}$ × 0.742 if female × 1.21 if Afro-American)[10] or the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[11] Creatinine clearance was estimated using the Cockcroft-Gault formula.[12] Complete blood count, urea, phosphate, and creatinine were measured by standard laboratory methods in the central laboratory of the hospital. We evaluated thrombin activity (prothrombin fragments 1+2, Enzygnost F1+2 micro, Dade Behring, Marburg, Germany), and the degree of plasmin generation (plasmin-antiplasmin complexes [PAP], Enzygnost PAP micro, Dade Behring) by the use of commercially available kits. Markers of endothelial injury (von Willebrand factor [vWF], thrombomodulin) were measured using the commercially available kits from American Diagnostica, Greenwich, Connecticut, United States, and adhesion molecules (inercellular adhesion molecule, vascular cell adhesion molecule [VCAM]) were measured using the kits from R&D Systems, Quantikine, Abingdon, United Kingdom. Tissue plasminogen activator (tPA) and its inhibitor, plasminogen activator inhibitor, were assayed using the kits from Bioopol, Umea, Sweden. High-sensitivity C-reactive protein (CRP) was measured using the commercially available assay from American Diagnostica and interleukin 6 (IL-6) using the commercially available kit from R&D Systems. Renalase was assessed using the commercially available

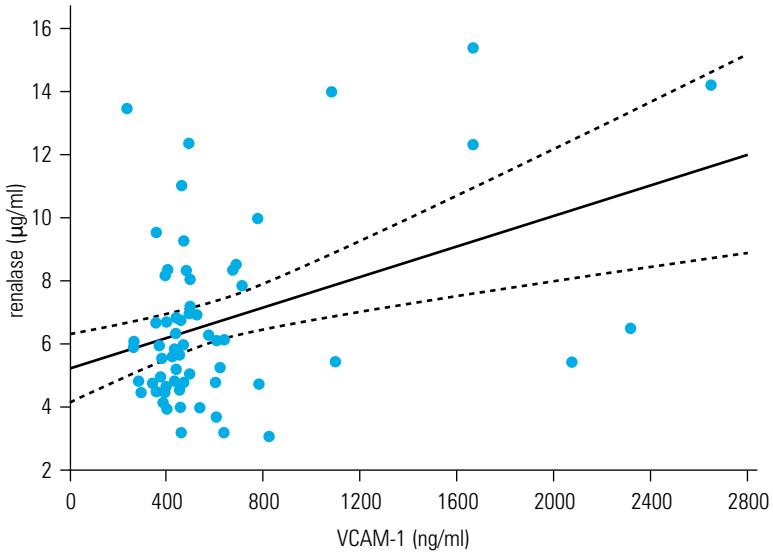


FIGURE 1 Correlation between renalase and vascular cell adhesion molecule-1 (VCAM-1) in kidney allograft recipients; $r = 0.40$, $P < 0.001$

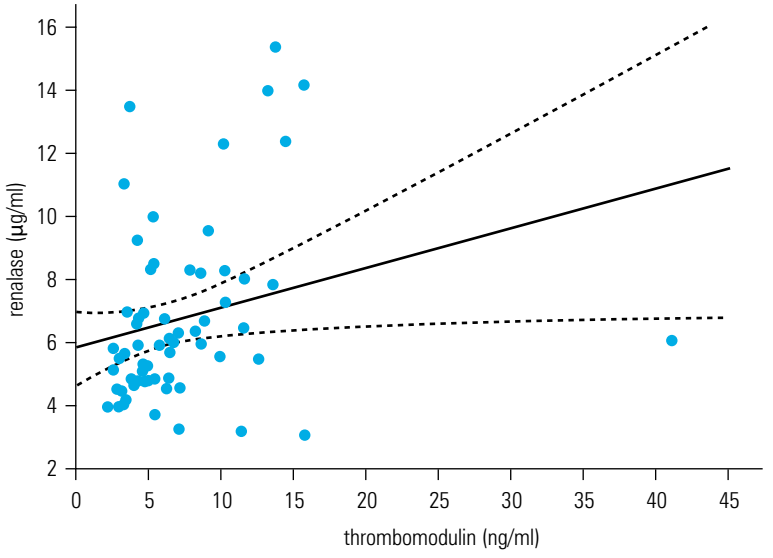
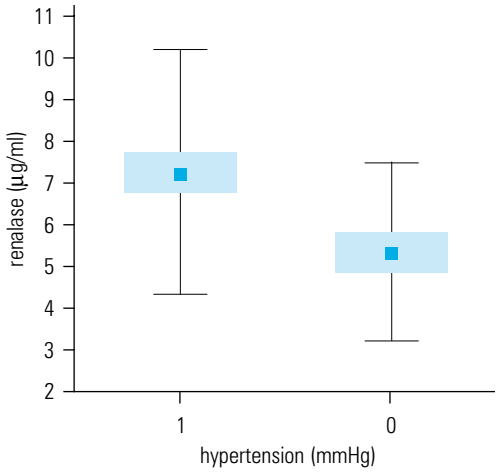


FIGURE 2 Correlation between renalase and thrombomodulin in kidney allograft recipients; $r = 0.25$, $P < 0.05$

FIGURE 3 Renalase in kidney allograft recipients in relation to hypertension; $P < 0.05$



kits from USCN Life Science Inc., Wuham, China. Healthy volunteers (n = 27) were included in the study to obtain normal ranges for renalase.

Statistical analysis Data were expressed as means ± standard deviation (SD). The data given were analyzed using the Statistica 7.0. computer software. Normal distribution of variables was analyzed using the Shapiro-Wilk's *W* test. The data were also logarithmically transformed to achieve normal distribution where possible. Normally distributed data are reported as mean ± SD, nonnormally distributed data are expressed as a median and minimal–maximal value. The Spearman or Pearson correlations were calculated as appropriate with $P < 0.05$ considered statistically significant. The multiple regression analysis was used to determine independent factors affecting dependent variables. Factors showing a linear correlation with renalase ($P < 0.1$) were included in the analysis.

RESULTS Clinical and biochemical data of kidney allograft recipients are presented in TABLE 1. The mean serum renalase level in kidney allograft recipients was significantly higher compared with the control group (6.72 ±2.86 µg/ml vs. 3.86 ±0.73 µg/ml, $P < 0.001$, respectively). In renal allograft recipients, renalase correlated with age ($r = 0.29$, $P < 0.05$), time after transplantation ($r = 0.34$, $P < 0.01$), CD44 ($r = 0.49$, $P < 0.0001$), VCAM ($r = 0.40$, $P < 0.001$) (FIGURE 1), serum creatinine ($r = 0.49$, $P < 0.001$), eGFR (by CKD-EPI formula, $r = -0.44$, $P < 0.0001$; by MDRD, $r = -0.43$, $P < 0.001$; by Cockcroft-Gault, $r = -0.39$, $P < 0.01$), serum phosphate ($r = 0.33$, $P < 0.05$), urea ($r = 0.41$, $P < 0.01$), CD146 (r=0.36, $P < 0.01$), vWF ($r = 0.26$, $P < 0.06$), thrombomodulin ($r = 0.26$, $P < 0.05$) (FIGURE 2) and tended to correlate with tPA ($r = 0.24$, $P = 0.05$). In a multiple regression analysis, renalase was predicted in 58% by serum creatinine (β value, 0.33, $P = 0.01$), F = 4.49, SE = 2.12, and $P < 0.00029$.

In patients with eGFR over 60 ml/min, renalase was significantly lower than in patients with eGFR below 60 ml/min (5.72 ±2.02 µg/ml vs. 7.63 ±3.22 µg/ml, $P < 0.001$). In hypertensive kidney allograft recipients, renalase was significantly higher than in normotensives (FIGURE 3).

DISCUSSION Even a successful kidney transplantation did not restore normal kidney function and the majority of kidney allograft recipients had stage 2 or 3 CKD.[13] It is known that CKD is related to increased cardiovascular mortality.[14] Chronic renal failure has also been associated with impaired immunity and subclinical inflammation involving cytokines derived from adipose tissue – adipocytokines. Endothelial cell injury is a common finding in patients after kidney transplantation as shown previously.[9] In humans, declining renal function may also affect the levels of inflammatory molecules, because serum CRP and IL-6 levels are inversely correlated with creatinine clearance.[15]

Worsening renal function may enhance overall inflammatory responses because of the decreased renal clearance of factors that are directly or indirectly involved in inflammation and endothelial dysfunction.

We observed that renalase was significantly higher in kidney transplant recipients than in healthy volunteers. In patients with eGFR over 60 ml/min, renalase was significantly lower than in patients with eGFR below 60 ml/min. In addition, renalase was related in the univariate analysis to kidney function, age, time after transplantation, and markers of endothelial dysfunction. However, in the multiple regression analysis, renalase was predicted independently by kidney function. Only one clinical study, by Schlaich et al.,[16] presented also in the abstract form, showed that in patients with resistant hypertension (n = 22) arterial renalase was significantly higher in normotensive control subjects (n = 4, $P < 0.05$). However, the whole body noradrenaline spillover tended to be higher in hypertensive patients, but without statistical significance ($P = 0.12$). Arterial renalase correlated negatively in univariate analysis with systolic blood pressure in the entire cohort ($r = -0.52$, $P < 0.05$).

The association between renalase and hypertension was also observed by Zhao et al.,[17] who were the first to show that in the Han Chinese population, the renalase encoding gene was a novel susceptibility gene for essential hypertension and its genetic variations might influence blood pressure. In Caucasian patients participating in the Heart and Soul Study, Farzaneh-Far et al.[18] reported that a functional missence polymorphism (C allele) in renalase (Glu37Asp) was associated with left ventricular hypertrophy, systolic and diastolic dysfunction, poor exercise capacity, and inducible ischemia; however, no association between this CC genotype and blood pressure in the population of stable coronary artery disease was observed. In a recent paper, Stec et al.[19] describes polymorphisms of renalase gene and their associations with hypertension in patients on hemodialysis. We observed previously that diastolic blood pressure together with kidney function were predictors of renalase in kidney allograft recipients,[20] but in the present study we could only notice that renalase was significantly higher in hypertensive kidney allograft recipients than in normotensives.

This is the first report regarding the correlations between renalase and endothelium injury markers; however, renalase levels were predicted by kidney function. It results from the fact that as kidney function deteriorates, endothelial damage increases, which is reflected by the rise in vWF, thrombomodulin, and cytokines. It should be emphasized that renalase is secreted not only by the kidney, but also by cardiomyocytes, liver, adipose tissue, skeletal muscles, endothelium, and the central nervous system.

In conclusion, renalase, highly elevated in kidney transplant recipients, is predominantly dependent on kidney function. Due to wide tissue distribution of renalase, its function may be organ-specific. Therefore, its synthesis and excretion might be related not only to kidney function. However, further studies are needed to prove a possible role of renalase in cardiovascular pathology, as well as in kidney allograft recipients and novel targeted therapies.

REFERENCES

1 Mancuso P, Antoniotti P, Quarna J, et al. Validation of a standardized method for enumerating circulating endothelial cells and progenitors: flow cytometry and molecular and ultrastructural analyses. Clin Cancer Res. 2009; 15: 267-273.

2 Wątorek E, Paprocka M, Duś D, et al. Endostatin and vascular endothelial growth factor: potential regulators of endothelial progenitor cell number in chronic kidney disease. Pol Arch Med Wewn. 2011; 121: 296-301.

3 Hogas S, Ardeleanu S, Segall L, et al. Changes in arterial stiffness following dialysis in relation to overhydration and to endothelial function. Int Urol Nephrol. 2011. [Epub ahead of print].

4 Klausen K, Borch-Johnsen K, Feldt-Rasmussen B, et al. Very low levels of microalbuminuria are associated with increased risk of coronary heart disease and death independently of renal function, hypertension, and diabetes. Circulation. 2004; 110: 32-35.

5 Mallamaci F, Tripepi G, Cutrupi S, et al. Prognostic value of combined use of biomarkers of inflammation, endothelial dysfunction, and myocardiopathy in patients with ESRD. Kidney Int. 2005; 67: 2330-2337.

6 Xu J, Li G, Wang P, et al. Renalase is a novel, soluble monoamine oxidase that regulates cardiac function and blood pressure. J Clin Invest. 2005; 115: 1275-1280.

7 Hennebry SC, Eikelis N, Socratous F, et al. Renalase, a novel soluble FAD-dependent protein, is synthesized in the brain and peripheral nerves. Mol Psychiatry. 2010; 15: 234-236.

8 Li G, Xu J, Wang P, et al. Catecholamines regulate the activity, secretion, and synthesis of renalase. Circulation. 2008; 117: 1277-1282.

9 Malyszko J, Malyszko JS, Pawlak K, Mysliwiec M. The coagulo-lytic system and endothelial function in cyclosporine-treated kidney allograft recipients. Transplantation. 1996; 62: 828-830.

10 Levey AS, Berg RL, Gassman JJ, et al. Creatinine filtration, secretion and excretion during progressive renal disease. Modification of diet in Renal Disease Study Group. Kidney Int. 1989; 36: S73-80.

11 Levey AS, Stevens LA, Schmid CH, et al.; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009; 150: 604-612.

12 Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976; 16: 31-41.

13 Malyszko J, Malyszko JS, Mysliwiec M. Serum neutrophil gelatinase-associated lipocalin correlates with kidney function in renal allograft recipients. Clin Transplant. 2009; 68: 681-686.

14 Gross P, Schirutschke H, Barnett K. Should we prescribe blood pressure lowering drugs to every patient with advanced chronic kidney disease? A comment on two recent meta-analyses. Pol Arch Med Wewn. 2009; 119: 644-647.

15 Pecoits-Filho R, Heimbürger O, Bárány P, et al. Associations between circulating inflammatory markers and residual renal function in CRF patients. Am J Kidney Dis. 2003; 41: 1212-1218.

16 Schlaich M, Socratous F, Eikelis N, et al. Renalase plasma levels are associated with systolic blood pressure in patients with resistant hypertension. J Hypertens. 2010; 28: e437.

17 Zhao Q, Fan Z, He J, et al. Renalase gene is a novel susceptibility gene for essential hypertension: a two-stage association study in northern Han Chinese population. J Mol Med. 2007; 85: 877-885.

18 Farzaneh-Far R, Desir GV, Na B, et al. A functional polymorphism in renalase (Glu37Asp) is associated with cardiac hypertrophy, dysfunction, and ischemia: data from the heart and soul study. PLoS One. 2010; 5: e13 496.

19 Stec A, Semczuk A, Furmaga J, et al. Polymorphism of the renalase gene in end-stage renal disease patients affected by hypertension. Nephrol Dial Transplant. 2011. [Epub ahead of print].

20 Malyszko J, Zbroch E, Malyszko JS, et al. Renalase, a novel regulator of blood pressure, is predicted by kidney function in renal transplant recipients. Transplant Proc. 2011, 43: 3004-3007.

ARTYKUŁ ORYGINALNY

Renalaza, czynność nerek i markery dysfunkcji śródbłonka u chorych po przeszczepieniu nerki

Edyta Zbroch, Jolanta Małyszko, Jacek Małyszko, Ewa Koc-Żórawska, Michał Myśliwiec

Klinika Nefrologii i Transplantologii, Uniwersytet Medyczny w Białymstoku, Białystok

SŁOWA KLUCZOWE

ciśnienie tętnicze, czynność nerek, przeszczepienie nerki, renalaza

STRESZCZENIE

WPROWADZENIE Renalaza jest enzymem wydzielanym przez nerki, który powoduje degradację krążących we krwi katecholamin, przez co może mieć wpływ na regulację ciśnienia tętniczego. U chorych po przeszczepieniu nerki często występuje dysfunkcja śródbłonka.

CELE Celem pracy była ocena zależności pomiędzy stężeniem renalazy w surowicy, ciśnieniem tętniczym a czynnością nerek u chorych po przeszczepieniu nerki.

PACJENCI I METODY Morfologia, stężenie mocznika i kreatyniny, lipidogram i glikemia na czczo zostały ocenione standardowymi metodami laboratoryjnymi. Oceniono również markery krzepnięcia: fragmenty protrombiny 1+2; markery fibrynolizy: tkankowy aktywator plazminogenu (*tissue plasminogen activator* – tPA), inhibitor aktywatora plazminogenu typu 1, kompleks plazmina–antyplazmina; markery funkcji/uszkodzenia śródbłonka: czynnik von Willebranda (*von Willebrand factor* – vWF), trombomodulinę, cząsteczki adhezyjne, cząsteczki adhezji komórkowej naczyń (*vascular cell adhesion molecule* – VCAM); markery stanu zapalnego: białko C-reaktywne oznaczone metodą o dużej czułości i interleukina 6. Stężenie renalazy oceniono za pomocą powszechnie dostępnego zestawu.

WYNIKI Średnie stężenie renalazy u chorych po przeszczepieniu korelowało z wiekiem, czasem po przeszczepieniu, sCD44, VCAM, stężeniem kreatyniny, oszacowanym przesączaniem kłębuszkowym (*estimated glomerular filtration rate* – eGFR; oszacowanym za pomocą wzorów: CKD-EPI, MDRD, Cockcrofta i Gault), stężeniem fosforanów, mocznika, sCD146, vWF, trombomoduliny oraz wykazywało tendencję do korelacji z tPA. U chorych z eGFR >60 ml/min stężenie renalazy było mniejsze niż u osób z niższym eGFR. U chorych po przeszczepieniu z nadciśnieniem tętniczym stężenie renalazy było większe niż u pacjentów bez nadciśnienia tętniczego. Analiza wieloczynnikowej regresji wykazała, że czynnikiem predykcyjnym poziomu renalazy było w 58% przypadków stężenie kreatyniny.

WNIOSKI Stężenie renalazy, znacznie zwiększone u chorych po przeszczepieniu nerki, w głównym stopniu zależy od czynności nerek, która pogarsza się z wiekiem i w miarę upływu czasu po przeszczepieniu. Istnieje potrzeba przeprowadzenia dalszych badań potwierdzających przypuszczalną rolę renalazy w patogenezie nadciśnienia tętniczego u chorych po przeszczepieniu nerki oraz jej ewentualnego wykorzystania jako celu terapeutycznego.

Adres do korespondencji:
dr med. Edyta Zbroch, Klinika Nefrologii i Transplantologii, Uniwersytet Medyczny, 15-540 Białystok, ul. Żurawia 14, tel.: 85-740-94-64, fax: 85-743-45-86, e-mail: edzbroch@poczta.onet.pl

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