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Searching for Perfect Marker - Differences and Dependencies in Serum Levels of Renalase, KIM-1, MCP-1, Calbindin and GST-Pi in Patients with Diabetes, Glomerulonephritis and Congenital Defects of the Kidney.

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Abstract

Diagnosis of kidney diseases has recently become more comprehensive and accurate by using new renal markers. Despite the fact that creatinine and cystatin c have been sufficient in determining kidney function, they did not indicate the exact site of the damage and they were often insufficient in predicting the course of the disease. Aim of the study was to evaluate the potential correlations and differences in levels of six factors related to kidney function and injury: kidney injury molecule-1 (KIM-1), ncalbindin (CALB), glutathione S-transferase Pi (GST-Pi), calbindin and monocyte chemoattractant protein-1 (MCP-1), between renal patients with diabetic nephropathy (DM), congenital defects (CD) of the kidney and glomerulonephritis (GN). Study involved 75 patients: 49 with diabetic nephropathy, 12 with congenital defects and 14 with glomerulonephritis. Levels of renalase was measured using immunoenzymatic tests. Levels of other markers: calbindin, glutathione-S-transferase (GST-pi), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1) and monocyte chemoattractant protein-1 (MCP-1), were analyzed using Kidney Toxicity-1 Panel and BioPlex system, designed for analyses in urine and optimized by us for serum.

From all analyzed markers, only levels of KIM-1 differed significantly between any subgroups, and that was for CD and DM. Renalase correlated significantly negatively with creatinine and positively with all other markers, apart from MCP-1. Obtained results indicate, that serum renalase, KIM-1, calbindin and GST-pi are related to kidney function, with KIM-1 being the most exact, while MCP-1 levels are unrelated to creatinine and glucose levels, does not differ between patients with diabetic nephropathy and other subgroups, and therefore seem to be independent of diabetes. Also, serum-optimized Kidney Toxicity Panel 1 kit for determination of selected markers gave results similar to previous ones and therefore the method can be valuable in determination of analyzed factors.

Key words: renalase; KIM-1; MCP-1; GST-pi; IL-18; calbindin; glomerulonephritis; diabetic nehropathy; congenital defects of the kidney

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1. Introduction

Renal diseases of different etiology are characterized by multifactorial changes, not only in the kidneys. The kidney disease outcome is often also completely different; It is already known that this results not only from the fact of the damage itself, but also to a large extent from the stimulation of the immune system and changes in the cardiovascular system. Patients with diabetic nephropathy or glomerular kidney damage, together with patients with hypertensive nephropathy, represent the majority of people with chronic kidney disease. In turn, rare, congenital kidney defects represent a small percentage of patients with CKD, and the occurrence of the disease is in many cases independent of other diseases and style of life. However, it also leads to changes in immunity and haemodynamics, which measured using appropriate, adequate indicators, can have significant, added predictive value [1].

Diagnosis of kidney diseases has recently become more comprehensive and accurate by using new renal markers. Despite the fact that creatinine and cystatin c have been sufficient in determining kidney function, they did not indicate the exact site of the damage and they were often insufficient in predicting the course of the disease [2]. It has already been noted that in the case of many factors, the simultaneous analysis of several markers allows a much more effective assessment of the state of not only the kidneys, but also have a predictive value regarding the course of the disease. Modern technologies allow also to perform many analyzes simultaneously in one, small sample of material, so the analysis time is significantly shortened, and its value significantly greater. Described biomarkers: renalase (RNLS), calbindin-1 (CALB1, calbindin-D28k), glutathione-S-transferase-pi (GST-π, GST-pi), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1) and monocyte chemoattractant protein-1 (MCP-1), are found in serum and urine and serve as good indicators of many kidney dysfunctions as their levels change in response to inflammation and damage of certain part of nephron.

Renalase is a 37.8 kDa protein secreted by the proximal tubule, found both in serum and urine, with antihypertensive and cardioprotective properties. It is produced and released by kidneys, but also by many other organs, such as liver, brain, heart, intestine or skeletal muscles [3-5]. Primarily it was considered as monoamine oxidase, but the way of action of this protein still remains unclear; despite this, several studies *in vitro* and on animal models showed, that lack of renalase is associated with increased risk of many circulatory disorders, such as hypertension, myocardial necrosis or ventricular hypertrophy, and that kidney damage has a significant impact on levels of this protein in serum and urine [5]. It was also shown, that renalase prevents against ischemic acute kidney injury [6].

Interleukin-18 is a pro-inflammatory cytokine, produced mainly by macrophages, but also found in kidney cells such as mesangial or tubular epithelial cells [7]. It belongs to IL-1 family of

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cytokines and is widely described as serum and urinary marker of particular renal dysfunctions. In kidney transplant recipient, kidney IL-18 expression levels are strongly increased due to intragraft macrophages activity [8]; in urine, interleukin-18 is concerned to be an early marker of acute kidney injury and has predictive value in evaluating the risk of mortality in patients with acute respiratory distress syndrome staying in intensive care units [9].

Calbindin was described as a good urinary marker of distal renal tubules damage more than 20 years ago [19]. Since that time, not many studies concerned this calcium-binding protein as marker of kidney damage; in fact, serum levels of calbindin were described only for cardiac arrest patients and were found to be a good biomarker of damage caused by extracorporeal shock wave lithotripsy [10, 11].

GST-π is a member of glutathione-S-transferases family of homodimeric and heterodimeric enzymes with a broad spectrum of activity, including metabolizing reactive products of carcinogens. GST family divides into six subfamilies - alpha (GSTA), mu (GSTM), omega (GSTO), pi (GSTP), theta (GSTT) and zeta (GSTZ) [12]. GST, especially GST-π, are expressed in many tumors and vary depending on involved tissue. Serum glutathione-S-transferase-π is considered as a marker of many gastrointestinal diseases, including malignancies – gastric, esophageal, colonic and pancreatic cancers; GST-pi levels are also increased in other gastrointestinal diseases, such as acute and chronic hepatitis, gallbladder stones [13, 14]. In renal cell carcinoma, expression of two isoenzymes of GST: alpha and pi was observed [15]; α-GST is detected mainly in the proximal cells, while π-GST is observed in the distal parts [16]. Describing GST-pi as a marker of renal dysfunction, most studies are concerned on urinary levels of this molecule. In proteinuric patients, urinary excretion of GST pi correlates inversely with clearance of creatinine [17]. This increase in GST pi in urine in renal damage, shown also in studies on neonates, may occur due to systemic oxidative stress [18].

Monocyte chemoattractant protein-1 comes mainly from endothelia and macrophage like cells [19]. MCP-1 is a factor mostly related to heart and circulatory system diseases, such as atherosclerosis, coronary artery disease, ischemic stroke or myocardial infarction, where serum MCP-1 is significantly increased comparing to healthy individuals [20], what is probably caused by ischemic conditions that stimulate macrophage and lymphocyte infiltration of damaged area of the tissue. In renal diseases, monocyte chemoattractant protein-1 is widely described as a factor involved in diabetic nephropathy [21-23]; also, urinary MCP-1 in patients with diabetic nephropathy increases gradually according to the clinical stage of this disease (1=normoalbuminuric, 2=microalbuminuric, and stage 3=macroalbuminuric) showing, that this factor may worsen renal tubular function. In another study, performed using Infrared Motion Analyzer (IRMA), urinary MCP-1 levels also increased together with degree of albuminuria , but serum level of MCP-1 in all study groups was lower than detection limit of method used in the study (20pg/mL) [23].

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KIM-1 is one of the mostly and widely reported molecule strictly linked to several kidney dysfunctions and diseases. KIM-1 production in nephron is limited to proximal tubules, and seems to be one of the best urinary indicators of damage, acute kidney injury and drug-induced nephrotoxicity [24], while usefulness of serum levels of this molecule is debatable; no differences in serum concentration of KIM-1 were found between patients before and after cisplatin treatment, despite a substantial and nephrotoxicity-related increase in its concentration in urine [25], while in other studies, plasma KIM-1 levels were significantly associated with acute and chronic kidney injury, and predicted progression to ESRD in type I diabetes patients [26].

As is easy to see, some of the factors mentioned above are rarely analyzed in relation to the etiology and course of kidney diseases. There are also no reports in which they would be analyzed at the same time in the same sample. In our previous research [27], we used two sets for the determination of selected biomarkers of kidney damage in the urine: Kidney Toxicity Assay-1 and Assay-2, which after the modification of the method we used for the determination of serum. Assay-1 enables measurement of such factors, as calbindin, clusterin, GST-π, IL-18, KIM-1, and MCP-1, while Assay-2 is designed to measure the concentration of such factors as albumin, β2M, cystatin C, NGAL, osteopontin, and TFF-3. Both assays, after the modification of the method, were used for the determination of serum. Due to promising results, especially those on relationship between renalase and MCP-1 in the group of patients with glomerulonephritis and nephrotic syndrome, and the fact that renalase and MCP-1 were reported to be associated with diabetes mellitus, we decided that a group of diabetes patients should be also investigated for additional dependencies. In addition, the fact that the used by us multiplex method allows to evaluate levels of many other factors, it allows to significantly expand the previous, preliminary analysis.

Therefore, the study was an attempt to answer and solve the following issues: 1) to evaluate the potential differences in levels of analyzed factors: renalase, calbindin, GST-pi, IL-18, KIM-1 and MCP-1, between renal patients with diabetic nephropathy (DM), congenital defects (CD) of the kidney and glomerulonephritis (GN); 2) to asses dependencies between analyzed factors and correlations of analyzed markers with kidney function determined by levels of creatinine in serum 3) to compare obtained results on levels of 5 other factors: calbindin, GST-pi, IL-18, KIM-1 and MCP-1, to the previous ones, and assess the potential suitability of the method for serum determinations, basing on the concentration range of previously and presently obtained results.

2. Materials and methods

Study population

Study involved 75 patients of Department of Nephrology, Transplantology and Internal Diseases of Pomeranian Medical University in Szczecin, with diagnosed kidney disease: 49 with diabetic

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nephropathy (DM), 12 with congenital defects (CD) other than autosomal dominant polycystic kidney disease, and 14 with glomerulonephritis (GN). Study was approved by a local ethics committee and patients have given informed consent. The study was accepted by local Bioethical Commitee (Pomeranian MEdical University, Szczecin) in year 2013. Number of the decision is KB-0012/60/13.

Material collection and preparation

Blood samples were collected in the morning. Complete blood count was performed. All biochemical and immunochemical tests were performed in blood serum, obtained from venous blood using S-Monovette tubes (Sarstedt, Nümbrecht, Germany) with a clotting activator, centrifuged for 10 minutes at 1000 × g, and freezed until use.

Bochemical and immunoenzymatic assays

Basic biochemical parameters: concentration of creatinine, uric acid, glucose, calcium, albumin and total protein were determined with ready reagent kits (BioMaxima, Lublin, Poland). Concentration of renalase was measured using ELISA coated with monoclonal anti-renalase antibodies (Cloud-Clone Corp., Houston, Texas, USA).

Multiplex assay

Concentration of calbindin, GST-pi, IL-18, KIM-1 and MCP-1 were measured using Bio-Plex Pro™ RBM Kidney Toxicity Assay-1 and Bio-Plex 200 system and software (BioRad, Hercules, California, United States of America). Method is based on fluorescently dyed microspheres, and flow cytometry, what allows the quantification of several analytes in one sample.
We applied a method described by us in our previous research. Basing on literature data and our previous study, serum samples were diluted 4x (1:3) with sample diluent. Due to the fact that the measurements for each patient are made in one sample in one well, it was impossible to determine the concentration of clusterin, which is also included in the panel, but is abundant in the blood serum and would require a much stronger (about 1:1000) dilution.

Statistical analysis

Statistical analysis was performed using STATISTICA 12 program (StatSoft). Determination of the distribution of the continuous variables was performed using Shapiro-Wilk test. As most of variables were not normally distributed, a nonparametric tests were used. Differences between analyzed subgroups were evaluated using Kruskal-Wallis ANOVA. Correlations were evaluated using Spearman's correlation rank test.

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3. Results

Detailed descriptive data on levels of particular factors, together with evaluation of differences are presented in Table 1. None of the markers differed significantly between subgroups, apart from levels of KIM-1 between CD and DM groups.

Table 1. Analysis of biomarker levels and other biochemical parameters in the subgroups of patients with various diseases, together with statistical analysis of differences; Data are presented as mean ± SD (minimum–maximum). Note: KIM-1 levels were in the range of determination in serum of only 71 patients: 47 with DM, 11 with CD and 13 with GN.

	ALL (N=75; 39M, 36F)	DM (N=49; 24M, 25F)	CD (N=12; 5M, 7F)	GN (N=14; 10M, 4F)	P
Age (years)	62 ± 17 (18 - 88)	69 ± 12 (30 - 88)*	48 ± 22 (18 - 82)*	51 ± 12 (30 - 70)*	<0,01
Serum creatinine (mg/dL)	1.70 ± 0.83 (0.67 - 3.91)	1.88 ± 0.83 (0.71-3.9)*	1.26 ± 0.89 (0.67 - 3.7)*	1.46 ± 0.56 (0.73 - 2.9)	<0.01
HGB (mmol/L)	8.32 ± 1.35 (5.6 - 13.2)	7.86 ± 1.22 (5.60 - 13.20)*	9.21 ± 1.38 (7.5 - 11.8)*	9.19 ± 0.97 (8 - 11.3)*	<0.01
RBC (T/L)	4.81 ±1.51 (3.17 - 15.70)	4.42 ± 0.80 (3.17 - 7.40)*	6.11 ± 3.11 (4.11 - 15.7)*	5.06 ± 0.43 (3.99 - 5.6)*	<0.01
MCHC (mmol/L)	20.1 ±1.1 (13.1 - 22.4)	20.03 ± 1.25 (13.10 - 22.00)	20.26 ± 0.91 (19.2 - 22.4)	20.24 ±0.71 (19.2-22)	NS
Uric acid (mg/dL)	8.0 ± 1.9 (4.4 - 12.7)	8.18 ± 2.04 (4.80 - 12.70)	7.43 ± 1.64 (4.4 - 10.2)	7.83 ± 1.47 (5.7 - 10.4)	NS
Total protein (g/dL)	6.6 ± 0.6 (5.6 - 8.5)	6.41 ± 0.48 (5.60 - 7.60)*	7.18 ± 0.94 (6.2 - 8.5)*	6.55 ± 0.56 (5.6 - 7.4)	<0.05
Albumin (g/dL)	4 ±0.5 (3.1-5.3)	3.83 ± 0.33 (3.10 - 4.60) *	4.48 ± 0.54 (3.7 - 5.3)*	4.26 ± 0.29 (3.8 - 4.8)*	<0.01
Glucose (mg/dL)	126.3 ± 44.0 (78-295)	142 ± 47 (78.00 - 295.00)*	95 ± 7 (84 - 104)*	99 ± 13 (78 - 122)*	<0.01
Calcium (mg/dL)	9.91 ±0.43 (9.08-11.87)	9.85 ± 0.37 (9.08 - 10.80)	10.1 ± 0.68 (9.2 - 11.9)	9.95 ± 0.32 (9.25 - 10.3)	NS
Serum renalase (ug/mL)	86.23 ±27.11 (20.40-147.85)	91.52 ± 26.70 (38.52 - 147.90)	72.78 ± 26.47 (20.4 - 106.1)	79.24 ± 25.31 (32.16 - 129.2)	NS
Calbindin (ng/mL)	21.8 ±8.77 (10.73-8.77)	21.64 ± 5.95 (10.73 - 39.30)	23.52 ± 16.49 (11.37 - 73.7)	20.88 ± 8.57 (10.73 - 38.3)	NS
GST-pi (ng/mL)	50.39 ±20.51 (1.33-109.77)	49.86 ± 22.12 (1.33 - 109.80)	56.92 ± 19.95 (21.58 - 89.5)	46.69 ± 13.95 (21.21 - 71.2)	NS
IL-18 (pg/mL)	484 ±140 (250-930)	494 ± 127 (250 - 920)	496 ± 190 (260 - 930)	439 ± 135 (320 - 850)	NS

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	ALL (N=75; 39M, 36F)	DM (N=49; 24M, 25F)	CD (N=12; 5M, 7F)	GN (N=14; 10M, 4F)	P
KIM-1 (pg/mL)	80 ± 68 (10-390)	91 ± 78 (10 - 390)*	42.73 ± 32.59 (10.0 - 120.0)*	68.46 ± 35.32 (20.0 - 130.0)	<0.05
MCP-1 (pg/mL)	224 ± 85 (90-470)	225 ± 88 (90.0 - 460.0)	206 ± 73 (100 - 350)	237 ± 88 (120 - 470)	NS

Analysis of correlation in the whole group

Analysis of correlations revealed, that in the whole group (N=75), renalase correlated positively with age (r=0.36, P<0.05), serum creatinine (r=0.53, P<0.05), uric acid (r=0.39, p<0.05) and glucose (r=0.27, P<0.05). Renalase correlated negatively with HGB (r=-0.35, P<0.05) and albumin (r=-0.26, P<0.05). Correlations for renalase and other markers are shown in Table 2.

Table 2. Correlations between analyzed markers in the whole group of patients (N=73).

ALL	Renalase	Calbindin	GST-pi	IL-18	KIM-1	MCP-1
Renalase	1,00	0,24	0,25	0,26	0,37	-0,18
Calbindin	0,24	1,00	0,35	0,33	0,22	0,01
GST-pi	0,25	0,35	1,00	0,13	0,15	0,04
IL-18	0,26	0,33	0,13	1,00	0,24	-0,04
KIM-1	0,37	0,22	0,15	0,24	1,00	-0,19
MCP-1	-0,18	0,01	0,04	-0,04	-0,19	1,00

Correlations in disease-based subgroups

After dividing patients into subgroups (DM, GN, CD), in most cases renalase did not maintain correlations observed for the whole group, except moderate, positive correlation between renalase and calbindin (R=0.36, P<0.05), and renalase and KIM-1 (R=0.38, R<0.05) in group of patients with diabetic nephropathy (DM), and correlation between RNLS and IL-18 (R=0.59, p<0.05) in patients with glomerulonephritis (GN). For other factors, the following correlations were found: in the CD subgroup: calbindin and GST-pi (r=0.65, P<0.05), calbindin and KIM-1 (r=0.80, P<0.05), IL-18 and KIM-1 (r=0.62, P<0.05); in the GN subgroup: calbindin and KIM-1 (r=0.56, P<0.05); in the DM subgroup: calbindin and GST-pi (r=0.34, P<0.05) and calbindin and IL-18 (r=0.31, P<0.05).

Correlations with creatinine

Apart from mentioned negative correlation with renalase, creatinine correlated positively with calbindin (r=0.35, P<0.05), GST-pi (r=0.27, P<0.05) and KIM-1 (r=0.65, P<0.05). After division into subgroups, only some of mentioned correlations were maintained. In the 1) DM subgroup: correlation with renalase (R=0.57, P<0.05) and KIM-1 (R=0.65, P<0.05); 2) CD subgroup: correlation with calbindin (R=0.84,

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P<0.05), KIM-1 (R=0.88, P<0.05) and MCP-1 (R=-0.66, P<0.05); 3) in the GN subgroups, no significant correlations between creatinine and any of the analyzed factors were found.

4. Discussion

Renalase remains a very enigmatic molecule, with attributed variety of properties, including anti-inflammatory, cytokine-like activity, and enzymatic activity involved in the regulation of catecholamine levels in blood, and therefore blood pressure. Although it has been repeatedly demonstrated that its concentration changes significantly already in the initial stages of kidney disease, it is difficult to find a specific relationship and the cause of this phenomenon.

Analyzing differences in levels of renalase between patients with diabetic nephropathy, congenital defects and glomerulonephritis, we did not show any significant differences between these subgroups. In our previous work [27], in which we used a different renalase-specific ELISA kit, and where we examined patients with glomerulonephritis without accompanying symptoms, with associated hypertension or nephrotic syndrome, we made a similar observation, that renalase levels do not differ between patients with different kidney diseases and dysfunctions. The same observation applies to calbindin; Il-18 and KIM-1, which differed significantly between patients with glomerulonephritis (without hypertension and nephrotic syndrome) and patients with congenital defects (and hypertension), where both molecules levels were higher in patients with congenital defects than with glomerulonephritis. In present study, Il-18 levels in CD patients were also higher than in GN group than in patients with congenital defects, but this difference did not reach the statistical significance. What is interesting, KIM-1 levels differed significantly between patients with congenital defects and diabetic nephropathy and were higher in the first subgroup; KIM-1 levels in GN patients were almost half as much as in our previous study (68.46 ± 35.32 vs. 133.5 ± 75.26 pg/mL) and did not differ significantly from other subgroups.

Apart from observed differences between subgroups, obtained levels of calbindin, GST-pi, IL-18, KIM-1 and MCP-1 among all CKD patients were similar to those in previous research [27]. Comparing previous to present values in the whole CKD patients group: 28.8 ± 14.4 ng/mL vs 21.8 ± 8.77 ng/ml for calbindin, 49.9 ± 18.4 ng/ml vs. 50.39 ± 20.51 ng/ml for GST-pi, 466.6 ± 134.9 pg/ml vs 484.4 ± 139.8 pg/ml for IL-18, 65.9 ± 44.4 pg/ml vs. 79.72 ± 68.37 pg/ml for KIM-1 and 185.9 ± 77.9 pg/ml vs. 224.4 ±85.41 pg/ml for MCP-1 it can be concluded, that multiplex method is a potentially sufficiently accurate method and can be successfully used in the determination of these factors, of course after prior validation and standardization of the method.

Referring to correlations between other analyzed markers - CALB, GST-pi, IL-18, KIM-1 and MCP-1, there are only few scientific reports regarding such dependencies, but none of them are based on the method used in our research. MCP-1, similarly to our previous study conducted with this method, again did not correlate with any of the analyzed factors in the whole group of patients. However, due to the strong negative correlation between renalase and MCP-1 in patients with glomerulonephritis and nephrotic syndrome in previous research, as well as the potential contribution of these proteins to the development of diabetes, possible interactions between analyzed factors in patients with diabetes were assumed. Interestingly, DM was

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the only group in which no correlation between MCP-1 and any of the factors, including renalase and biochemical parameters, were observed. What is more, MCP-1 was the only factor for which no correlation with renalase was observed in the whole group of patients. Therefore, although the relationship between these factors and diabetes cannot be excluded, it seems that their way of action may be independent of each other. Lack of difference in MCP-1 levels between analyzed subgroups, among which was the diabetic subgroup also undermines mentioned in introduction, described in literature relationship with diabetes.

Analyzing other factors, GST-pi again correlated with calbindin and again, it was the only correlation found for GST-pi in the whole group. Therefore, such correlation should be analyzed in detail in further studies, as both factors are markers of function and injury of further parts of nephron - GST-pi secretion is limited to distal tubule, and calbindin - to distal tubule and collecting duct. In fact, GST-pi is a molecule from the family of glutathione s-transferases, which is given less attention in studies than the GST -alpha isoform;

Much more attention is devoted to urinary KIM-1 and IL-18, especially in the context of acute kidney injury (AKI). These factors, along with NGAL, are increasingly described urinary markers of acute kidney injury and many other kidney dysfunctions. It should be noted, that while in serum KIM-1 is also considered a kidney-specific marker, Il-18 is a cytokine involved in many immune reactions, and its level in serum should be also interpreted with caution, especially when it is analyzed solitary. As in our previous study, serum IL-18 and KIM-1 correlated with each other, and such relation was also found in present research, these factors can be an important element of prediction of severity of the disease ant its potential course, especially that IL-18 is a strong mediator of inflammation and increase in its concentration might make the disease worse. Analysis of this factors together may have a much more important diagnostic and clinical significance.

Although several years have passed since the discovery of renalase, both the method of measuring the levels of this protein, as well as the method of measurement of its activity, together with results of such measurement, are still an issue. There are many interpretative problems caused by the fact that the level of this molecule in serum and urine of healthy individuals, as well as in nephrological and cardiac patients, determined by different methods, differ significantly depending on the test used. We decided to carry out the experiment using different than before, the most commonly used test for immunoenzymatic determinations of the level of this molecule. Thanks to this we can refer to both the results obtained by us and the results of other researchers. As expected, there is a huge discrepancy between obtained results; it also probably affects the obtained correlations of renalase with other factors.

The mean concentration of renalase measured in our previous research by different reagent kit was about 800-times lower than in present study (108.4 ± 76.8 ng/ml vs. 86.23 ± 27.13 ųg/ml, respectively) [27]. Such difference, even if such situation should not take place, is the result of using test with different sensitivity and accuracy, but comparing some literature data, even using the same test, very different results can be obtained.

Some measurements of renalase levels in serum/urine, performed using less popular reagent kits, showed results which oscillated around hundreds of ng/mL. Gluba-Brzózka et al. (2014) showed, that in healthy subjects renalase levels in serum were 251.0 ± 157.0 ng/ml, and in chronic kidney disease patients - 316.1 ± 155.3 ng/ml, and that this difference is statistically significant; they also divided patients into

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subgroups basing on the stage of the disease and observed, that among them, only the difference between stage I-II (3554.5± 1821.6 ng/ml) and stage V (dialysed, 369.0 ± 110.5 ng/ml) was statistically significant [28]. Similar, but a bit lower levels were described by Oguz EG et al. (2016); It was shown, that in hemodialysis patients serum renalase levels are significantly higher than in control group (212 ± 127 ng/ml vs. 116 ± 67 ng/ml, respectively; $p<0.001$) [29]. There is also a report on plasma levels of renalase in hemodialysed men, in which mean renalase levels were just 20.59 ± 32.63 ng/ml, which deviates significantly from the mentioned above results [30]. Moreover, Elcioglu OC et al. (2015) showed, that in patients with simple renal cyst, unlike most results and tendencies, renalase levels are lower than in healthy individuals (130.3 ± 60.6 ng/ml vs. 160.4 ± 57.6 ng/ml; $p<0.05$) [31]. Also, in 2014, Wang F et al. showed, that renalase in CKD patients differs between patients with I-II and III-V stages of the disease (162.1 ± 40.1 ng/l vs. 217.4 ± 103.8 ng/l, respectively; $p<0.05$), but there is no significant difference between the stages I-II subgroup and the control group, in which the mean renalase levels were 167.8 ± 69.4 ng/L; it is also worth noting that renalase levels are presented as ng/l, and that is again a different concentration range [32].

I [3, 28-39].

There is also a series of studies indicating, that renalase concentration in serum is much higher and reaches ug/mL. Some scientific research results show, that in healthy individuals renalase levels are about 3.86 ± 0.73 ug/ml [3], 3.98 ± 0.79 [40] or 4.00 ±1.37 ug/mL [35]; in kidney transplants - 6.72 ± 2.86 ug/ml [3], and 6.6 ± 2.78 ug/ml [34]; in hemodialyzed patients: 27.53 ± 9.39 ug/ml [35] and 25.98 ± 8.12 ug/ml [37], and patient on hemodiaiftration - 17.87 ± 6.91 ug/ml [37]. There is also a study on pediatric renal patients, concerning concentration of renalase in single-kidney group and reference group, where levels of this protein were 23.07 (19.96, 27.22) ug/mL and 26.75 (22.64, 29.20, ug/ml, respectively, and this difference was statistically significant [39].

Considering all of the mentioned results, it is difficult to compare our results with any of them. The mean concentration we have obtained - 86.23 ± 27.11 ug/ml, is relatively high and about 4x higher when compared to those found in CKD patients in other studies. We can only conclude that, as in many other publications, significant positive correlation with creatinine levels indicates that the concentration of RNLS changes significantly with changes in renal function [3, 33, 41]. However, it still does not determine whether this decrease in glomerular filtration, or injury of any other part of the nephron increases the amount of renalase produced and released, or whether there is an reverse relationship. The fact that in our experiment renalase correlated with all markers associated with potential kidney damage, with the exception of MCP-1, strengthens the theory that it is closely related to the condition of the kidneys, but its concentration is regulated by still unknown mechanism

5. Conclusions

Lack of significant differences between subgroups for most factors: renalase, NGAL, calbindin, GST-pi and MCP-1 indicates that their level can not be a differentiating factor in laboratory analysis. Lack of correlation between serum creatinine and IL-18 or MCP-1 suggests, that their concentration does not result directly or exclusively from changes in kidney function and do not relate to the type and range of damage. It

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seems that the already widely used KIM-1 demonstrates its effectiveness both as a marker of damage and as a potential differentiating factor. Relating to serum levels of KIM-1, in addition to the widely reported indicatory properties of urinary form of this molecule, it also appears to be sensitive to small changes in kidney function described by serum creatinine and therefore eGFR. Its significantly higher mean concentration in serum of DM patients when compared to CD, and non-significant when compared to GN patients indicates the possible relationship between serum levels of this factor and the occurrence or course of diabetes mellitus.

Moreover, similar to the results we obtained earlier among patients with glomerulonephritis and , renalase does not differ between patients with different kidney diseases; therefore it is an indicator of damage independent of etiology, which makes it only a potential marker of damage, but not of renal function. The use of different sets and individuals excludes renalase as a precise marker of changes in renal function, however, it does not discriminate RNLS as a marker while analyzing proportions, differences and correlations with other markers and between different groups of patients. Still, there is a significant problem in establishing a precise and comparable to other parameters method of determination of renalase levels and range, which requires the unification and determination of reference values.

In addition, the Kidney Toxicity Panel used, although designed for urine tests, gave results similar to those obtained in previous research and to some of the literature data, hence it seems to be a good, relatively inexpensive and fast test for accurate determination of the concentration of selected markers in one, small serum sample.

Limitations

Due to the fact that the determination of renalase levels in healthy subjects and chronic kidney disease patients have been repeatedly carried out, as well as the number of possible analyzes was methodically limited, no control group was introduced in the study. This decision was also influenced by the fact that, according to most literature data, renalase levels in serum of CKD patients are still significantly higher than in healthy people.

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Authors Contributions

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Conflicts of Interest

Authors declare no conflict of interest.

Bibliografia

1. Wieliczko, M., P. Kulicki, and J. Matuszkiewicz-Rowinska, [Classification, epidemiology and etiology of chronic kidney disease]. Wiad Lek, 2014. **67**(3): p. 393-6.
2. Simões e Silva, A.C., et al., Interactions between Cytokines, Congenital Anomalies of Kidney and Urinary Tract and Chronic Kidney Disease. Clin Dev Immunol, 2013. **2013**.
3. Zbroch, E., et al., Renalase, kidney function, and markers of endothelial dysfunction in renal transplant recipients. Pol Arch Med Wewn, 2012. **122**(1-2): p. 40-4.
4. Desir, G.V. and A.J. Peixoto, Renalase in hypertension and kidney disease. Nephrol Dial Transplant, 2014. **29**(1): p. 22-8.
5. Matoszka, N., M. Wisniewska, and B. Dolegowska, *[The mysterious properties of renalase]*. Postepy Biochem, 2014. **60**(1): p. 90-3.
6. Wang, L., et al., Renalase prevents AKI independent of amine oxidase activity. J Am Soc Nephrol, 2014. **25**(6): p. 1226-35.
7. Wu, H., et al., IL-18 Contributes to Renal Damage after Ischemia-Reperfusion, in J Am Soc Nephrol. 2008. p. 2331-41.
8. Wyburn, K., et al., Macrophage-derived interleukin-18 in experimental renal allograft rejection. Nephrol Dial Transplant, 2005. **20**(4): p. 699-706.
9. Parikh, C.R., et al., Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the intensive care unit. J Am Soc Nephrol, 2005. **16**(10): p. 3046-52.
10. Hasegawa, S., et al., Increased levels of calbindin-D in serum and urine from patients treated by extracorporeal shock wave lithotripsy. J Urol, 1993. **149**(6): p. 1414-8.
11. Usui, A., et al., Neural tissue-related proteins (NSE, G0 alpha, 28-kDa calbindin-D, S100b and CK-BB) in serum and cerebrospinal fluid after cardiac arrest. J Neurol Sci, 1994. **123**(1-2): p. 134-9.
12. Nebert, D.W. and V. Vasiliou, Analysis of the glutathione S-transferase (GST) gene family. Hum Genomics, 2004. **1**(6): p. 460-4.
13. Niitsu, Y., et al., Serum glutathione-S-transferase-pi as a tumor marker for gastrointestinal malignancies. Cancer, 1989. **63**(2): p. 317-23.
14. Aliya, S., P. Reddanna, and K. Thyagaraju, Does glutathione S-transferase Pi (GST-Pi) a marker protein for cancer? Mol Cell Biochem, 2003. **253**(1-2): p. 319-27.
15. Grignon, D.J., et al., Glutathione S-transferase expression in renal cell carcinoma: a new marker of differentiation. Mod Pathol, 1994. **7**(2): p. 186-9.
16. de Geus, H.R., M.G. Betjes, and J. Bakker, Biomarkers for the prediction of acute kidney injury: a narrative review on current status and future challenges. Clin Kidney J, 2012. **5**(2): p. 102-108.
17. Branten, A.J., et al., Urinary excretion of glutathione S transferases alpha and pi in patients with proteinuria: reflection of the site of tubular injury. Nephron, 2000. **85**(2): p. 120-6.
18. Tsukahara, H., et al., Quantitation of glutathione S transferase-pi in the urine of preterm neonates. Pediatr Int, 2005. **47**(5): p. 528-31.
19. Kim, J.S., et al., Expression of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 after focal cerebral ischemia in the rat. J Neuroimmunol, 1995. **56**(2): p. 127-34.
20. Arakelyan, A., et al., Serum Levels of the MCP-1 Chemokine in Patients With Ischemic Stroke and Myocardial Infarction. Mediators Inflamm, 2005. **2005**(3): p. 175-9.
21. Banba, N., et al., Possible relationship of monocyte chemoattractant protein-1 with diabetic nephropathy. Kidney Int, 2000. **58**(2): p. 684-90.
22. Tashiro, K., et al., Urinary levels of monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8), and renal injuries in patients with type 2 diabetic nephropathy. J Clin Lab Anal, 2002. **16**(1): p. 1-4.
23. Morii, T., et al., Association of monocyte chemoattractant protein-1 with renal tubular damage in diabetic nephropathy. J Diabetes Complications, 2003. **17**(1): p. 11-5.
24. Malyszko, J., et al., Biomarkers of delayed graft function as a form of acute kidney injury in kidney transplantation. Sci Rep, 2015. **5**: p. 11684.
25. Tekce, B.K., et al., Does the kidney injury molecule-1 predict cisplatin-induced kidney injury in early stage? Ann Clin Biochem, 2015. **52**(Pt 1): p. 88-94.
26. Sabbisetti, V.S., et al., Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. J Am Soc Nephrol, 2014. **25**(10): p. 2177-86.
27. Serwin, N.M., et al., Serum levels of 12 renal function and injury markers in patients with glomerulonephritis. Pol Arch Med Wewn, 2016. **126**(7-8): p. 483-93.

28. Gluba-Brzozka, A., et al., Markers of increased cardiovascular risk in patients with chronic kidney disease. Lipids Health Dis, 2014. **13**: p. 135.
29. Oguz, E.G., et al., Increased serum renalase in hemodialysis patients: is it related to left ventricular hypertrophy? Ren Fail, 2016. **38**(8): p. 1180-6.
30. Dziedzic, M., et al., Relationship between renalase and N-terminal pro-B-type natriuretic peptide (NT pro-BNP) in haemodialysis patients. Ann Agric Environ Med, 2014. **21**(1): p. 132-5.
31. Elcioglu, O.C., et al., Renalase: Another puzzle piece between hypertension and simple renal cysts? Int Urol Nephrol, 2015. **47**(7): p. 1181-6.
32. Wang, F., et al., Serum renalase is related to catecholamine levels and renal function. Clin Exp Nephrol, 2014.
33. Malyszko, J., et al., Renalase, a novel regulator of blood pressure, is predicted by kidney function in renal transplant recipients. Transplant Proc, 2011. **43**(8): p. 3004-7.
34. Koc-Zorawska, E., et al., VAP-1, a novel molecule linked to endothelial damage and kidney function in kidney allograft recipients. Kidney Blood Press Res, 2012. **36**(1): p. 242-7.
35. Koc-Zorawska, E., et al., Vascular adhesion protein-1 and renalase in regard to diabetes in hemodialysis patients. Arch Med Sci, 2012. **8**(6): p. 1048-52.
36. Zbroch, E., et al., Renalase, a novel enzyme involved in blood pressure regulation, is related to kidney function but not to blood pressure in hemodialysis patients. Kidney Blood Press Res, 2012. **35**(6): p. 395-9.
37. Malyszko, J., et al., Renalase Is Removed By Kidneys And During Dialysis - Excess Related To CKD Complications?, in Curr Vasc Pharmacol. 2014.
38. Quelhas-Santos, J., et al., Plasma and urine renalase levels and activity during the recovery of renal function in kidney transplant recipients. Exp Biol Med (Maywood), 2014. **239**(4): p. 502-8.
39. Taranta-Janusz, K., R. Roszkowska, and A. Wasilewska, Renalase Levels in Children with Solitary Functioning Kidney. Indian Pediatr, 2015. **52**(12): p. 1047-50.
40. Malyszko, J., et al., Renalase is removed by kidneys and during dialysis - excess related to CKD complications? Curr Vasc Pharmacol, 2015. **13**(1): p. 134-40.
41. Wang, F., et al., Renalase might be associated with hypertension and insulin resistance in Type 2 diabetes. Ren Fail, 2014. **36**(4): p. 552-6.