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The Correlation between Albuminuria and Cystatin C in the Assessment of the Kidney Function in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Introduction: Cystatin C has shown a better correlation with albuminuria in relation to creatinine. It filters in glomeruli, reabsorbs in tubules, and does not go back into circulation, which makes it a reliable parameter for the assessment of kidney function.

Aim of the study: To determine albuminuria and serum cystatin C in patients with type 2 diabetes mellitus (DM) and to examine the correlation between these two parameters depending on the degree of albuminuria.

Patients and Methods: Forty-seven patients with type 2 DM were divided in three groups: Group I-25 patients with albuminuria < 30 mg/24 hours, Group II-15 patients with albuminuria of 30-299 mg/24 hours and Group III -7 patients with albuminuria > 300 mg/24 hours. For the purpose of assessing the kidney function, cystatin C and creatinine in the serum were determined and the glomerular filtration rate (GFR) was calculated.

Results: Higher degrees of albuminuria were associated with longer duration of disease, poorer regulation of glycaemia and blood pressure and kidney damage. The mean values of cystatin C were increased with the degree of albuminuria: 0.99(0,7-1,25;IQ) vs. 1.18(1,05-1,36;IQ) vs.1.74(1,45-2,01;IQ) mg/l. Cystatin C has shown a statistically significant direct correlation with the albuminuria, especially in the third group ($r= 0.82$, $p < 0.001$).

Conclusion: In this research, cystatin C has shown a good correlation with albuminuria. The values of cystatin C were associated with the changes in the values of albuminuria.

Key words: diabetes mellitus, cystatin C, albuminuria

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Introduction

Determination of albuminuria represents a screening method for establishing diabetic nephropathy. Kidney damage is present in 25-40% patients with type 2 diabetes mellitus

(DM) and together with hypertension it represents a major cause of end stage renal disease.^{1,2} The classification on microalbuminuria (albuminuria in the range of 30-299 mg/24 hours) and macroalbuminuria (albuminuria values

>300 mg/24 hours) is no longer in use in the present day which favours the concept of persistent albuminuria for the values ranging from 30-299 mg/24 hours and > 300mg/24 hours.³ The normal values of albuminuria are <30 mg/24 hours. The presence of albuminuria ranging from 30-299 mg/24 hours in patients with type 2 DM indicates kidney damage that is caused by DM and described under the term diabetic nephropathy.^{1,3}

Physical activity, infection, hyperglycaemia and hypertension can all have an impact on the secretion of albumins through urine. Therefore, it is necessary to repeat examinations two or three times in the subsequent 3-6 months, once the presence of albuminuria has been determined, in order to be able to safely confirm the occurrence of kidney damage.³ Given that kidney damage can occur in patients with type 2 DM without albuminuria, it is necessary to determine serum creatinine and calculate the glomerular filtration rate (GFR) regardless of the stage of albuminuria.³ Creatinine values are influenced by age, gender, nutrition and constitution. Deficiencies of these methods gave birth to the tendency to find a more reliable parameter for the assessment of kidney function. Cystatin C is a relatively more recent parameter; it filters in glomeruli, reabsorbs in tubules and does not go back into circulation. Due to such characteristics it is a reliable indicator of kidney function.⁴⁻¹⁰ The application of cystatin C has proved sound in the assessment of the initial damage of the kidney function when the values of creatinine are still within the reference values, that is, in the area of the "blind range" of creatinine.^{4,11} An increase in cystatin C in patients suffering from DM who do not have albuminuria was observed, which is explained by associating cystatin C with subclinical damage of tubules, while the presence of albumins in urine indicates damage of glomeruli.¹² By applying different equation it is possible to calculate the values of GFR from the serum cystatin C.^{2,13} Cystatin C has shown a better correlation in relation to creatinine when the methods of "golden standard" for determining GFR were used.¹³ Its application is recommended in the conditions where creatinine is less precise (e.g. in obese persons, children and elderly people).⁶⁻¹¹

Aim of the study

The aim of the study was to determine albuminuria and serum cystatin C in patients with type 2 DM, and to examine the connection of these two parameters depending on the degree of albuminuria.

Patients and Methods

This prospective study included 47 patients with type 2 DM, aged 40-70, who were hospitalized at the Department of Endocrinology, Diabetes and Metabolic Diseases of the University Hospital-Clinical Centre in Banja Luka. Depending on the degree of albuminuria, the patients

were divided in three groups: Group I-25 patients with albuminuria < 30 mg/24 hours, Group II-15 patients with albuminuria ranging from 30-299 mg/24 hours and Group III-7 patients with albuminuria > 300 mg/24 hours. The study was approved by the institution's ethics committee. Due to the possible influence on the values of cystatin C in serum, we excluded patients with acute diabetic complications, thyroid disease (hypothyroidism and hyperthyroidism), malnutrition, heart decompensation, inflammation signs, and receiving corticosteroid therapy.^{5, 8, 14,15} Biochemical blood and urine analyses were done at the Institute of Laboratory Diagnostics of the University Hospital-Clinical Centre in Banja Luka. The blood and urine samples were taken in the morning. The duration of DM, presence of comorbidities and current medications were determined by anamnestic and medical documentation. Albumins in the urine were determined by the turbidimetric method (Integra 400+, Roche). Cystatin C levels were determined by the immunoturbidimetric method (Cobas 6000, Roche). Using an electronic calculator, the GFR for creatinine was calculated using MDRD formula, and for cystatin C using CKD-EPI formula. $GFR (MDRD) = (ml/min/1.73m^2) = 186 \times creatinine^{-1.154} (\mu mol/l) \times age^{-0.203} (\times 0.742 \text{ if female})$. $GFR (CKD-EPI) = 76.7 \times cystatin C^{-1.19}$.¹⁶ Systolic and diastolic blood pressure was measured with a standard mercury sphygmomanometer (Welch Allyn) before the physical examination. Hypertension was defined as systolic blood pressure > 140/80 mmHg, according to the guidelines of the American Diabetes Association (ADA) of 2015.³ The serum creatinine using a buffered kinetic Jaffe reaction without deproteinization; blood glucose by enzymatic method with hexokinase and glycosylated hemoglobin (HbA1c%) using the test of inhibition of latex agglutination were measured on the AU 680, Olympus. The body mass index (BMI) for the assessment and state of nutrition follow up was calculated according to Quetelet's formula as body weight in kilograms divided by height in meters squared (kg/m²).

Statistical analysis, tabular and graphical results were performed using Microsoft Office Word 2007, Microsoft Excel 2007 and the SPSS for Windows (version 21.0). All the data was processed using standard procedures of descriptive statistics. The X^2 test was used to compare the difference between groups and the Pearson's parametric correlation was used for test of correlations between different variables. To compare the mean values of characteristics we used the Student's t-test for independent samples and the Mann-Whitney U-test for two independent samples. $P < 0.05$ was considered significant.

Results

The main demographic, clinical and laboratory characteristics are shown in Table 1. The average values of fasting glucose concentration, HbA1c and diastolic blood pressure were above the recommended in all three groups of

examinees, without any statistical significance between groups. For regulation of glycaemia, 28 patients were treated with insulin, 15 patients with oral antihyperglycemic drugs and 4 with combinations of oral drugs and insulin.

Table 1. Demographic, clinical and laboratory data on examinees according to the degree of albuminuria

Parameter	Group I	Group II	Group III	All examinees	Statistical significance
Number of examinees	25	15	7	47	p<0.05
Age, years	61.34(58.06-64.14; IQ)	63.1(60-67.52;IQ)	60.12(57-61.3;IQ)	60.18(57.15-61.9;IQ)	ns
Duration of DM, years	8.8(7.9-9.5;IQ)	9.4(8.1-10.1;IQ)	13.3(11.5-14.6;IQ)	10.25(8.9-12.5;IQ)	p <0.05
BMI, kg / m2	28.04(26.4-29.5;IQ)	27.57(26.8-29;IQ)	28.75(27.5-30.1;IQ)	28.02(26.9-29;IQ)	ns
SBP, mmHg	133.79 (127.6-138.68)	134.5 (128.14-140.5;IQ)	141.66(133.56-146.1;IQ)	134(128-139.5;IQ)	ns
DBP, mmHg	93.1 (90,61-96.5;IQ)	94.5 (91.5-97.8;IQ)	93.5(91-98.8;IQ)	92.73(89.5-95.7;IQ)	ns
Glycaemia, mmol/l	10.87 (7.9-12.5;IQ)	11.04 (8.9-13.1;IQ)	11.8(9.5-13.6;IQ)	11.43(9-12.8;IQ)	ns
HbA1c, %	9.37(7.7-10.06;IQ)	9.42(8.1-10.2;IQ)	9.8(9-11.5;IQ)	9.63(7.9-9.3;IQ)	ns
Creatinine, μ mol/l	87.89(66-95.5;IQ)	89.9 (70-98.7;IQ)	159.6(125.4-87.3;IQ)	96.5(88.5-124.3;IQ)	p<0.05
Cystatin C, mg/l	0.99 (0.7-1.25;IQ)	1.18(1.05-1.36;IQ)	1.74(1.45-2.01;IQ)	1.13(0.95-1.28;IQ)	p<0.05
GFR by MDRD equation, ml/min/1.73m ²	82.60(77-99;IQ)	75.62(64.5-88.9;IQ)	53.60(49.5-60.7;IQ)	73.04(66.5-91.87;IQ)	p<0.001
GFR by CKD-EPI equation, ml/min/1.73m ²	81.25(77.5-93.5)	73.54(60.5-87.6;IQ)	52.3(48.1-59.4;IQ)	71.52(68.5-88.7;IQ)	p<0.001

BMI - body mass index; SBP- systolic blood pressure, DBP-diastolic blood pressure; HbA1c- hemoglobin A1c; GFR- glomerular filtration rate, MDRD- *Modification of Diet in Renal Disease*; CKD-EPI- *Chronic Kidney Disease- Epidemiology*; X- mean value; StD- standard deviation; ns- not significant

The mean creatinine level were elevated only in the third group, with a statistically significant difference between the groups (p <0.05). The mean cystatin C level were elevated in the second and third groups, with high statistically significant difference between the groups (p <0.001). In the third group the mean value of GFR according to MDRD and CKD-EPI equation was <60 ml/min/1.73m².

The values of cystatin showed a statistically significant direct correlation with albuminuria values in the second group (r = 0.22, p <0.05), Figure 1, and in the third group this relationship was directly and statistically highly significant (r = 0.82, p <0.001), Figure 2.

Figure 1. Correlation between cystatin C and albuminuria in group II, p<0,05

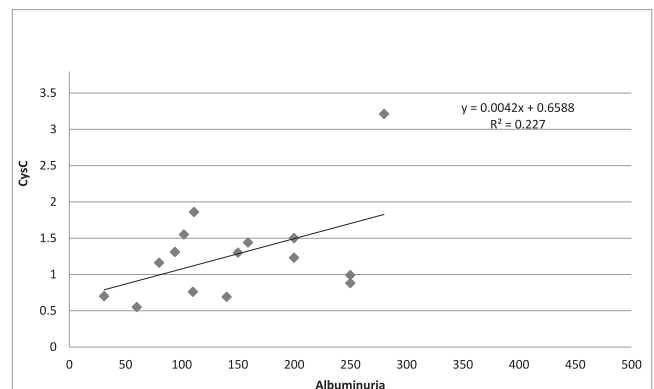
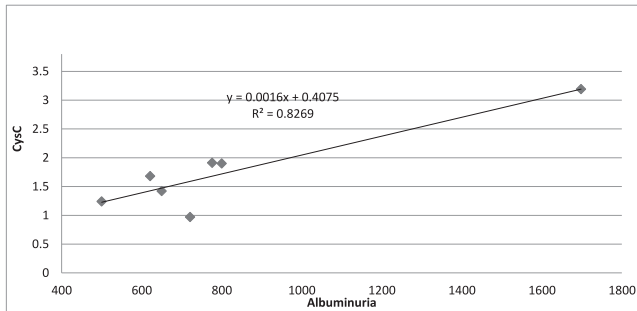


Figure 2. Correlation between cystatin C and albuminuria in group III, $p < 0,001$



The values of creatinine showed a statistically significant direct correlation with albuminuria in the third group ($r = 0.52$, $p < 0.05$). The values of cystatin C were not in statistically significant correlation with age, sex and BMI.

Discussion

The number of patients with DM in the world is rising dramatically and unexpectedly, and the term pandemic is being used more and more when talking about this disease.¹ Diabetes mellitus type 2 makes 80-90% of all patients with DM.^{1,3} The leading causes of chronic kidney disease (CKD) are DM and hypertension. According to the recommendations, assessment of kidney functions in patients with type 2 DM should be done immediately after the diagnosis. Timely assessment of kidney function enables detection of patients with kidney damage in early stage when treatment can slow down the progression of kidney damage.^(1-3, 17) Considering that patients with DM can have kidney function damage without the presence of albuminuria, it is necessary to determine the serum creatinine and calculate GFR.⁽³⁾ In our study, the subjects were divided into three groups, according to the degree of albuminuria. Serum cystatin C and creatinine were determined in all patients for the assessment of kidney function and GFR was calculated based on these two parameters. We examined the correlation between cystatin C, as a marker of early damage of kidney function and albuminuria.

Hyperglycemia and hypertension represent the main factors of the risk of microvascular complications in DM.³ In all three of our groups, glycemic control, evaluated on the fasting glucose and HgA1c, as well as diastolic blood pressure, were higher than recommended. In other authors' data, these values were closer to the target values.^{11,12,17,18.}

The highest degree of albuminuria, which was present in the third group of examinees, was accompanied by elevated levels of creatinine and cystatin C, as well as reduced GFR values, indicating the presence of CKD. In other authors' data, it can also be seen that, with a significant degree of albuminuria, other parameters which indicate the status

of kidney function, such as cystatin C and creatinine, were elevated.^{11, 12, 19} In the second group of patients, albuminuria values indicate the presence of initial nephropathy. In this group, the values of cystatin C, unlike creatinine, were elevated. GFR values were lower and indicated a slightly impaired kidney function. It is known that the normal values of creatinine in serum can be maintained as long as there is no significant kidney function damage (loss of up to 50% of normal). Elevated levels of cystatin C and normal serum creatinine values in patients in the second group show that cystatin C is sensitive to changes in the "creatinine-blind range".¹¹ These results can be found in other authors. These results showed a higher degree of sensitivity and specificity of cystatin C compared to creatinine in the assessment of kidney function.^{11, 17, 18} In the first group of patients, the values of albuminuria, creatinine and cystatin C were within reference ranges. This group of patients needs better glycemia control and blood pressure so that the kidney function is protected as long as possible.

Determining the correlation between albuminuria and cystatin C has shown that there is a direct and statistically significant relationship between these two parameters. This correlation increased with increasing degree of albuminuria. The correlation between albuminuria and creatinine was significant only in the third group, when there was already a significant kidney damage ($p < 0.05$). These results suggest that cystatin C may play a role in detecting early damage of kidney function that cannot be diagnosed by serum creatinine. Other authors have also shown that serum cystatin C, unlike creatinine, accurately reflects changes in albuminuria.^{11, 12, 19}

Conclusion

The results in this study have shown that there is significant correlation between cystatin C and albuminuria even at a lower degree of albuminuria, when there is still no correlation between albuminuria and creatinine. The cystatin C measurement is a simple and practical method for determining present damages expressed with different degrees of albuminuria.

References

1. KDOQI Clinical Practice Guideline for Diabetes and CKD: 2012 update.[database on the Internet]. New York: National Kidney Foundation. Available from: http://www.kidney.org/professionals/KDOQI/guidelines_diabetesUp/diabetes-ckd-update-2012.pdf Accessed March 15, 2013.
2. Arora S, Renal function in diabetic nephropathy. *W J Diab* 2010; 1(2):48-56. <http://dx.doi.org/10.4239/wjd.v1.i2.48> PMID:21537427 PMID:PMC3083882
3. American Diabetes Association. Standards of Medical Care in Diabetes-2015. *Diabetes Care* 2015;38(Suppl 1):S58-S66. PMID:25537710
4. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Int* 2013; 3(Suppl 1):S5-14.

5. Newman DJ. Cystatin C. *Ann Clin Biochem* 2002; 39:89-104. <http://dx.doi.org/10.1258/0004563021901847> PMID:11928770
6. National Kidney Foundation. Cystatin C, New York. Available at: <http://www.kidney.org/professionals/tools/pdf/CystatinC.pdf>. Accessed February 10, 2013.
7. Brguljan PM, Cimerman N. Human Cystatin C. *Turk J Biochem* 2007; 32(3):95-103.
8. Fassett RG, Venuthurupalli S.K, Gobe GC, Coombes JS, Matthew A. Biomarkers in chronic kidney disease: a review. *Kidney Int* 2011; 80(8):806-21. <http://dx.doi.org/10.1038/ki.2011.198> PMID:21697815
9. Walczak K, Rosniak-Bak K, Paradowski M, Moczulski D. Cystatin C as a marker of renal function in diabetic kidney disease. *Diabet Dosw Klin* 2009; 9,2:69-72.
10. Horng H.C, O'Riordan S.E, Webb M.C, Stowe H.J, Simpson D.E, Kandarpa M, Coakley A.J et al. Cystatin C improves the detection of mild renal dysfunction in older patients. *Ann Clin Biochem* 2003; 40:648-55. <http://dx.doi.org/10.1258/000456303770367243> PMID:14629803
11. Yoo J.S, Lee Y.M, Lee E.H, Kim J.W, Lee S.Y, Jeong K-C et al. Serum Cystatin C reflects the progress of albuminuria. *Diabetes Metab J* 2011; 35:602-9. <http://dx.doi.org/10.4093/dmj.2011.35.6.602> PMID:22247903 PMCid:PMC3253971
12. Jeon YK, Kim MR, Huh JE, Mok JY, Song SH, Kim SS et al. Cystatin C as an early biomarker of nephropathy in patients with type 2 diabetes. *J Korean Med.Sci* 2011; 26:258-63 <http://dx.doi.org/10.3346/jkms.2011.26.2.258> PMID:21286018 PMCid:PMC3031011
13. Beauvieux M.C, Le Moigne F, Lasseur C, Raffaitin C, Parlemoine C, Barthe N et al. New predictive equations improve monitoring of kidney function in patients with diabetes. *Diabetes Care* 2007;30(8):1988-1914. <http://dx.doi.org/10.2337/dc06-2637> PMID:17536079
14. Bokenkamp A, Van Wil A.E, Lentze M.J, Stoffel-Wagner B. Effect of corticosteroid therapy on serum cystatin C and β 2-Microglobulin concentrations. *Clin Chem* 2002; 48:1123-6. PMID:12089191
15. Fricker M, Wiesli P, Brandle M, Schwegler B, Schmid C. Impact of thyroid dysfunction on serum cystatin C. *Kidney Int* 2003; 63:1944-7. <http://dx.doi.org/10.1046/j.1523-1755.2003.00925.x> PMID:12675875
16. National Kidney Foundation. Calculator For Health Care Professionals. New York. Available at: http://www.kidney.org/professionals/kdoqi/gfr_calculator.cfm. Accessed October 30, 2013.
17. Pucci L, Triscornia S, Lucchesi D, Fotino C, Pellegrini G, Pardini E et al. Cystatin C and estimates of renal function: searching for better measure of kidney function in diabetic patients. *Clin Chem* 2007;53:480-88. <http://dx.doi.org/10.1373/clinchem.2006.076042> PMID:17259236
18. Hojs R, Bevc S, Ekart R, Gorenjak M, Puklavec M. Serum cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of kidney function. *Neph Dial Transplant* 2006; 21: 1855-62. <http://dx.doi.org/10.1093/ndt/gfl073> PMID:16524933
19. Wasen E, Isoaho R, Mattila K, Vahlberg T, Kivela SL, Irjala K. Renal impairment associated with diabetes in elderly. *Diabetes Care* 2004;27(11):2648-53 <http://dx.doi.org/10.2337/diacare.27.11.2648> PMID:15505000

Korelacija albuminurije i cistatina C u procjeni oštećenja bubrežne funkcije kod oboljelih od diabetes mellitus tip 2

SAŽETAK

Uvod: Cistatin C pokazao je bolju korelaciju sa albuminurijom u odnosu na kreatinin. Filtrira se u glomerulima, reapsorbira u tubulima, ne vraća u cirkulaciju, te zbog ovoga predstavlja pouzdan parametar za procjenu bubrežne funkcije.

Cilj rada: Odrediti albuminuriju i serumski cistatin C kod oboljelih od DM tipa 2, ispitati korelacije ova dva parametra u zavisnosti od stepena albuminurije.

Ispitanici i metode: Četrdeset i sedam ispitanika oboljelih od DM tipa 2 su u zavisnosti od stepena albuminurije podijeljeni u tri grupe: grupa I-25 ispitanika sa albuminurijom < 30 mg/24 sata, grupa II-15 ispitanika sa albuminurijom od 30-299 mg/24 sata i grupa III-7 ispitanika sa albuminurijom > 300 mg/24 sata. Za procjenu bubrežne funkcije, pored cistatina C određivan je serumski kreatinin, te izračunavana jačina glomerularne filtracije (JGF).

Rezultati: Veći stepen albuminurije bio je udružen sa dužim trajanjem bolesti, slabom regulacijom glikemije i krvnog pritiska, te oslabljenom bubrežnom funkcijom. Prosječne vrijednosti cistatina C su se povećavale sa stepenom albuminurije: 0.99(0,7-1,25;IQ) vs. 1.18(1,05-1,36;IQ) vs.1.74(1,45-2,01;IQ)mg/l. Cistatin C je pokazao statistički značajnu direktnu korelaciju sa vrijednostima albuminurije, naročito u trećoj grupi ($r = 0,82$, $p < 0,001$).

Zaključak: U ovom istraživanju, cistatin C je pokazao dobru korelaciju sa albuminurijom u procjeni bubrežne funkcije kod oboljelih od DM. Vrijednosti cistatina C su pratile promjene u vrijednostima albuminurije.

Ključne riječi: diabetes mellitus, cistatin C, albuminurija