URINARY CLUSTERIN CONCENTRATIONS - A POSSIBLE MARKER OF NEPHROPATHY? PILOT STUDY.

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Background: Clusterin is a glycoprotein which participates in a number of pathophysiological processes in the organism. Information about clusterin use in the diagnosis of nephropathy and the differential diagnosis of proteinuria has been published recently.

Aim: Search for correlations between urinary clusterin concentration and other renal function markers. Evaluation of urinary clusterin measurement use in the differential diagnosis of nephropathy.

Methods: Urea, creatinine, IgG, transferin, Na, K in serum and 24-hour collected urine were measured in a sample of 82 individuals. Cystatin C in sera was also measured as were GMT, α -1 microglobulin, albumin, total protein in urine. In all probands urinary clusterin was assayed (ELISA).

Results: Urinary clusterin values correlated with urinary total protein concentrations ($r = 0.28$; $p = 0.018$), total protein/creatinine index ($r = 0.26$; $p = 0.02$). No correlation was found between urine clusterin concentration and glomerular filtration rate, age, urine GMT/creatinine, α -1-microglobulin, urine albumin and albumin/creatinine ratio or Na, K fractional excretions. We found no urinary clusterin differences by sex of probands.

No evidence of any relationship between urine clusterin and presence of defect of renal function, number of risk factors ($\chi^2 = 16.0$; $DF = 15$; $p = 0.38$), albumin/creatinine index ($\chi^2 = 0.76$; $DF = 3$; $p = 0.86$), total protein/creatinine ($\chi^2 = 6.5$; $DF = 3$; $p = 0.09$), GMT/creatinine ($\chi^2 = 2.3$; $DF = 3$; $p = 0.51$), high urinary α -1-microglobulin ($\chi^2 = 4.1$; $DF = 3$; $p = 0.25$) or decreased of GFR ($\chi^2 = 1.3$; $DF = 3$; $p = 0.74$).

Conclusions: A positive correlation exists between urinary clusterin and urinary total protein and total protein/creatinine index. Urinary clusterin measurement with ELISA test does not offer any advantage over routinely used parameters for nephropathy diagnosis and the differential diagnosis of proteinuria type.

INTRODUCTION

The differential diagnosis of proteinuria is an essential component in the diagnosis of renal diseases. Proteinuria may be caused by increased capillary permeability to proteins, with excess tubule capacity for back resorption (glomerular proteinuria), disorders in back resorption of filtrated proteins (tubular proteinuria), extreme increase in some blood proteins (prerenal proteinuria) or inflammatory and cytolytic processes in the urinary tract (postrenal proteinuria). The diagnostic examination of proteinuria is made by determining the urinary protein/creatinine index, urine electrophoresis, measurement of albumin and urinary albumin/creatinine index, calculation of selectivity proteinuria index, determination of α -1-microglobulin, light chain immunoglobulins and urinary paraproteins.

Recent information suggests the possible importance of other potential diagnostic urinary parameters.

A number of papers have been published on the possible use of urinary clusterin concentration analysis in the assessment of type proteinuria and renal function disorders⁵⁻⁶.

METHODS

The study tested 82 individuals after obtaining their informed consent. The probands had suspect nephropathy of various etiology (49 persons with suspect early diabetic nephropathy, 5 individuals with primary glomerulopathy, 28 subjects with nephropathy due to chronic pyelonephritis).

To evaluate the presence and severity of nephropathy, the following scheme of minimally three risk parameters was used: a) decreased GF value according to MDRD (Modification of Diet in Renal Disease)⁷ < 1.1 ml/s, b)

Table 1. Basic statistical parameters (normality evaluated with Komolgorov-Smirnov test)

Parameter	Units	Mean	Median	SD	Normality
BMI	-	28.5	28.0	7.1	No
U-Protein	g/day	0.62	0.25	0.95	No
U-Protein/U-Creatinine	g/mmol	97.6	36.5	153.5	No
U-Clusterin	kIU/l	79.2	50.0	135.6	No
FE K	%	12.2	9.5	11.2	No
FE Na	%	1.4	1.2	0.99	No
U-Creatinine	mmol/day	12.7	11.5	7.8	No
GFR Cystatin C	ml/s	1.4	1.2	0.8	No
U-GMT/U-Creatinine	nkat/mmol	82.5	74.2	45.4	No
Selectivity proteinuria index	-	0.41	0.34	0.22	No
GFR MDRD	ml/s	1.1	1.0	0.4	No
Number of risk factors	-	3.1	3.0	1.2	No
S-Cystatin C	mg/l	1.22	1.1	0.5	No
S-Creatinine	umol/l	118.8	110.0	55.2	No
S-Transferin	g/l	2.1	2.2	0.6	Yes
S-Immunoglobulin G	g/l	9.9	10.1	2.8	Yes
U-Albumin	mg/day	297.8	81.6	619.5	No
U- α -1-microglobulin	mg/day	18.5	10.6	27.2	No
U-Albumin/U-Creatinine	mg/mmol	49.0	10.7	94.8	No
U-GMT	nkat/l	0.6	0.5	0.5	No
U-IgG	mg/l	0.05	0.01	0.11	No
U-Transferin	mg/l	0.01	0.01	0.01	No
Age	years	66.3	68.0	16.0	No

SD – standard deviation.

Table 2. Quartiles of urine clusterin and presence of nephropathy

Presence of defect	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Suma %
Defekt No	15	6	0	0	25.9
Defekt Yes	38	18	2	2	74.1
Suma %	65.4	29.6	2.5	2.5	100.0

$$\chi^2 = 1.6; DF = 3; p = 0.67$$

enhanced urinary albumin/creatinine index > 2.5 g/mol in men and > 3.5 g/mol in women, c) enhanced urinary α -1-microglobulin > 10 mg/l, d) enhanced urinary GMT/creatinine > 94.6 nkat/mmol, c) enhanced urinary protein/creatinine > 20 g/mol.

The probands did not need to observe any special diet before sample collection. Laboratory parameters were determined in samples of venous blood and urine collected for 24 hours. Biologic material samples were centrifuged within 60 min after collection (10 min at 2000 g and 4° C), then analyzed and kept at -80° C for at least 10 days. In blood and urine we determined urea (Advia 1650), creatinine (Jaffe, Advia 1650), IgG (Advia 1650), transferin (Advia 1650), Na and K (Advia 1650), serum cystatin C

(Advia 1650). Only in urine were measured GMT (Advia 1650), α -1-microglobulin (Advia 1650), albumin (Advia 1650), total protein (Advia 1650). In all probands urinary clusterin was assayed by the ELISA method (BioVendor Laboratory Medicine, Czech Republic; limit of detection 50 kU/l, repeatability in three levels in 8 replicates < 6 %, intermediate precision < 9 %, urine stability without preservative agents at -20 °C for at least one month). In addition a selectivity proteinuria index was calculated⁸ and glomerular filtration rate was assessed from serum cystatin C⁸.

To compare the studied parameters, the group of probands was divided according to clusterin assessment into quartiles. The presence of other measured risk pa-

Fig. 1. Interactive dot diagram for evaluation of the diagnostic efficacy of urine clusterin measurement

parameters were also calculated as their overall number and then statistically processed.

The data were processed statistically by means of the MedCalc program (Belgium). Values of individual parameters were expressed as means $\pm$ standard deviation and medians. Correlations of individual parameters were studied using a Spearman correlation coefficient (by data distribution). A value of $p < 0.05$ was considered statistically significant. The data were evaluated for correlations between clusterin levels (measurable vs. non-measurable) and other studied parameters (over vs. below a limit of detection) and using a chi-squared test (χ^2 -test).

RESULTS

Most data showed an abnormal distribution. Only 28 probands (34 %) had higher clusterin values than the limit of detection (50 kU/l) (Tab 1).

Urinary clusterin values correlated with urinary total protein concentrations ($r = 0.28$; $p = 0.018$), total protein/creatinine index ($r = 0.26$; $p = 0.02$). No correlation was found between urine clusterin concentration and glomerular filtration rate, age, urine GMT/creatinine, α -1-microglobulin, urine albumin and albumin/creatinine ratio or Na, K fractional excretions. We found no urinary clusterin differences by sex of probands.

Using a χ^2 -test there was no clear evidence of relationship between urine clusterin and presence of defect of renal function (see above) (Tab 2), number of risk factors ($\chi^2 = 16.0$; $DF = 15$; $p = 0.38$), albumin/creatinine index ($\chi^2 = 0.76$; $DF = 3$; $p = 0.86$), total protein/creatinine ($\chi^2 = 6.5$; $DF = 3$; $p = 0.09$), GMT/creatinine ($\chi^2 = 2.3$; $DF = 3$; $p = 0.51$), high urinary α -1-microglobulin ($\chi^2 = 4.1$; $DF = 3$; $p = 0.25$) or decreased of GFR ($\chi^2 = 1.3$; $DF = 3$; $p = 0.74$).

The division of patients according to severity of renal dysfunction showed diagnostic insufficient diagnostic ef-

ficacy of urine clusterin (AUC 0.55; 95 % CI 0.44-0.66; LR +1.8, sensitivity of clusterin 35 % and specificity 81 %) (Fig 1).

DISCUSSION

Clusterin (70–80 kDa; synonym ApoJ) is a glycoprotein involved in many physiological and pathophysiological processes. Two clusterin forms exist: the first (secretory, exogenous) occurs in biological fluids; the second form (endogenous) is contained in the cytoplasm and nucleus⁴. Secretory clusterin originates from preclusterin (60 kDa), which is subsequently cleaved into two chains of mature protein (alpha, beta). This form has antiapoptotic and antiangiogenic effects²⁻⁴. Endogenous clusterin originates by alternative cleaving of preclusterin; the final molecule acts as a strong stimulator of apoptosis⁴.

Clusterin effects can be summarized as follows: secretory clusterin acts as a cytoprotective extracellular chaperon and its expression and synthesis are increased in individuals suffering from various forms of stress and especially in patients with malignant tumors¹⁻⁴.

Extracellular clusterin plays a number of roles, e.g. in the production of amyloid in Alzheimer's disease, binding abnormal prions, "heat shock proteins" and reduced degradation of many components of the extracellular matrix. Clusterin is also involved in immune processes regulation, lipid transport, sperm maturation and protection of cell membranes⁴.

The results of experimental studies indicate the pathophysiological importance of clusterin particularly in the pathogenesis of glomerulonephritis, polycystic kidneys, renal tubular diseases, neurodegenerative disorders, atherosclerosis, myocardial infarction and malignant tumors⁴.

As the data are still sporadic and often contradictory, the aim of the present pilot study was to determine relations between urinary clusterin concentration and other parameters of renal functions in urine, as well as assess the use of clusterin measurement in the differential diagnosis of proteinuria.

With regard to the presence and diagnostic importance of urinary clusterin there is ambiguity and numerous discrepancies. Several years ago, the potential importance of serum clusterin was written in the diagnosis of renal disorders; many patients with renal disorders had lower urinary clusterin, which was considered as one cause of renal diseases (e.g. clusterin deficiency leads to activation of complement cascade and thus to possible renal injury)⁵⁻¹¹. On the other hand, there is experimental evidence that individuals with impaired renal parenchyma or toxic injury have higher urinary clusterin concentrations (together with other proteins)^{6, 10, 12}. This is interesting apropos clusterin production by a stressed cell; enhanced urinary clusterin levels could be permanent and independent of diuresis changes or renal perfusion^{4, 6}.

Our findings confirm significant correlations between urinary clusterin and total protein and total protein/creatinine index, but no unambiguous proof was found for

correlations of measured clusterin values with enhanced parameters in urine. We also failed to confirm the assumption that clusterin determination more significantly correlated with tubular proteinuria than glomerular proteinuria. It should be mentioned here that clusterin in the organism is enhanced nonspecifically under extreme cell stress. These reasons could be limiting for the generalized diagnostic effectiveness of urinary clusterin in the diagnosis of nephropathy.

It can be concluded that despite the proved existence of a positive correlation between urinary clusterin and urinary total protein, urinary clusterin measurement does not confirm any evident advantage to routinely used parameters for nephropathy diagnosis and the differential diagnosis of proteinuria type.

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