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CLINICAL PATHOPHYSIOLOGY OF PROTEINURIA

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Abstract

Proteinuria is the most important classical criterion of renal pathology, and its main characteristic is considered to be the quantitative degree of protein excretion. The latter in most patients determines the degree of nephron damage, the chronicity of the process in the kidneys, the prognosis of nephrological disease and the term of development of renal failure. Therefore, the clinical significance of proteinuria is quite significant, and consists in the timely detection and adequate treatment of proteinuria. **The aim** of the study was to determine and compare proteinuria under conditions of daily and induced salt diuresis. **Materials and methods.** 20 patients with proteinuria were selected for the study, 10 patients with diabetes mellitus and 10 patients with hypertension, who were inpatients. The age of the patients ranged from 31 to 59 years. **Conclusions.** 1. With water-salt loading, glomerular filtration rate, diuresis and total proteinuria increase. 2. Determination of latent proteinuria is a more informative indicator of kidney damage than comparing protein excretion during spontaneous, daily and induced water-salt diuresis. 3. The ratio of specific proteinuria to excretion of mmol creatinine or 1 ml of glomerular filtrate in % during salt loading compared to spontaneous daily diuresis is an important criterion for the degree of kidney damage. 4. Specific proteinuria is a marker of nephron damage, since it shows the true amount of protein that the nephron loses under conditions of its functional provocation; therefore, the study of specific proteinuria is quite relevant in the preclinical stages of kidney damage and can serve as one of the screening tests in risk groups with urinary system pathology.

Keywords: Proteinuria; diabetes; kidney

Proteinuria is the most important classical criterion of renal pathology, and its main characteristic is considered to be the quantitative degree of protein excretion [1]. The latter in most patients determines the degree of nephron damage, the chronicity of the process in the kidneys, the prognosis of nephrological disease and the term of development of renal failure [3]. Therefore, the clinical significance of proteinuria is quite significant, and consists in the timely detection and adequate treatment of proteinuria.

Diagnostic proteinuria is considered to be the presence of protein in daily urine of more than 50 g for children and more than 150 mg for adults. By anatomical origin, proteinuria can be extrarenal (orthostatic, caused by physical exertion, fever, not exceeding 500 mg/day in adults) and renal, which is determined by the predominant lesion of the nephron structure (glomerular, tubular and mixed). By the degree of protein excretion, proteinuria is divided into microproteinuria - up to 1 g/day (in the presence of diabetes mellitus - up to 300 mg/day), moderate proteinuria - 1-2.5 g/day and macroproteinuria (nephrotic) - more than 2.5 g/day.

Microalbuminuria is an important sign of kidney damage, since the excretion of protein in the urine is also a nephrotoxin - a marker of future progression of kidney disease, which requires drug correction [4].

The first barrier to protein penetration into the urine is the basement membrane, which acts as a “molecular” sieve, i.e. only low-molecular proteins (primarily albumins and transferrin) can pass through the basement membrane in a healthy person. The negative charge of the glomerular capillary wall, which contains a large amount of sialic acids, is also important. This is due to the fact that proteins that also have a negative charge have a reduced chance of “passing” through the capillary filter. The next factor that is important for protein penetration through the glomerular filter is hemodynamic, since it has been proven that with an increase in blood flow velocity, conditions appear that contribute to increased permeability for protein.

It should be taken into account that the total surface area of the glomerular capillaries is quite large and equal to the body surface area. Given that the permeability of the glomerular capillaries is 20-50 times higher than that of the capillaries of other tissues, ultrafiltration occurs in the glomeruli and up to 75 g of protein per day enters the primary urine (provided that up to 180 ml of glomerular filtrate is formed per day). However, subsequently, the process of reverse reabsorption of water and the main components in the tubules occurs. Almost all the protein that ended up in the primary urine is absorbed in the proximal tubules [1]. This process occurs by the mechanism of pinocytosis, and therefore only the remnants of the protein that was not subject to the pinocytosis process enter the final urine.

So, as it was said, proteinuria can be glomerular and tubular, its magnitude is also determined by the glomerular filtration rate. However, it should be noted that the intensity of glomerular filtration can change with functional loads, therefore, most studies of proteinuria at rest may not detect protein loss and do not allow us to judge the functional capabilities of the nephron in terms of protein transport and the degree of nephron damage..

More than 20 years ago, the results of the first clinical studies were published, which indicated that a slight increase in urinary protein excretion, not determined by conventional clinical methods, such as sulfasalicylic, reflects a significant increase in the risk of irreversible damage to kidney function, primarily in patients with diabetes mellitus and essential hypertension. In the future, this phenomenon began to be called microalbuminuria [3]. UIA, being a reliable early sign of diabetic and hypertensive nephropathy, reflects a significant deterioration in the overall prognosis, associated primarily with a very high probability of cardiovascular complications [5, 8].

Along with 24-hour proteinuria, in some countries the ratio of urinary albumin to creatinine concentration is more often used [2, 9].

Low albuminuria can be considered one of the most reliable markers of worsening prognosis in the general population. Based on data obtained in the Framingham Offspring study [5], in individuals without hypertension and diabetes, an increase in the urinary albumin/creatinine ratio by one standard deviation within the range of low albuminuria is associated with a 37% increased risk of cardiovascular complications and a 55% increased risk of death.

The determination of low albuminuria values is a promising approach to assessing the long-term prognosis in patients with so-called pre-hypertension [12], as well as in other situations where the likelihood of developing hypertension is high, for example, in individuals with abdominal obesity or in the presence of aggravating family history.

While indicating early stages of kidney damage, for example, in essential hypertension, albuminuria simultaneously indicates the maximum likelihood of chronic heart failure, acute myocardial infarction, and cerebral stroke. In turn, in the case of already developed cardiovascular complications, persistent proteinuria indicates an unfavorable short- and long-term prognosis. It has also been established that in the presence of microalbuminuria in patients with acute myocardial infarction, the incidence of pulmonary edema increases almost 5 times, the incidence of ventricular arrhythmias increases more than 3.5 times, and mortality increases almost tenfold [11]. Similar results were obtained when assessing the prognostic value of microalbuminuria in cerebral strokes. The mortality of patients with microalbuminuria was more than 7 times higher than that of patients without increased urinary protein excretion. [12].

Proteinuria always corresponds to changes in the structure of the renal glomerulus, but they can be detected only with the help of special morphological methods of research, but not light

microscopy. Thus, as protein excretion increases in patients with type 2 diabetes mellitus, electron microscopy reveals a significant increase in the thickness of the glomerular basement membrane and the volume of mesangiocytes. Higher values of the thickness of the basement membrane were associated with increased serum concentrations of acute phase markers - C-reactive protein, serum amyloid A (SAA), interleukin-6 and fibrinogen [7]. At present, the relationship between proteinuria and deterioration of renal filtration function should be considered established. The most convincing arguments in favor of its existence were obtained during large prospective studies that included patients with hypertension and/or DM2. According to the NORDIL study, which included 10,881 patients with hypertension, the relationship between proteinuria, decreased glomerular filtration rate, which was assessed using Cockroft-Gault and MDRD, is reliable and independent. Patients with signs of kidney damage (albuminuria, decreased estimated glomerular filtration rate) were significantly more likely to reach the primary endpoint (fatal and non-fatal acute myocardial infarction, stroke, cardiovascular death) [10].

At the same time, the relationship between changes in glomerular filtration and proteinuria values in healthy and nephrologically ill patients under different physiological renal modes remains unstudied: daily and induced diuresis (water or water-salt). Meanwhile, fluctuations in filtration and reabsorption that occur in such patients can be a source of important information about the functional state of the kidneys.

That is, the aim of the study was to determine and compare proteinuria under conditions of daily and induced salt diuresis.

Materials and methods. 20 patients with proteinuria were selected for the study, 10 patients with diabetes mellitus and 10 patients with hypertension, who were inpatients. The age of the patients ranged from 31 to 59 years. The glomerular filtration rate in all patients was 53-128 ml/min. All patients were characterized by an elevated blood pressure: systolic 145±20 and diastolic 95±15 mm Hg. Protein excretion was determined in all patients during spontaneous daily diuresis and during water-salt induced diuresis with a 0.5% sodium chloride solution in an amount of 0.5% of body weight. The choice of the value of 0.5% of body weight is due to the fact that this load, as shown by previous studies, on the one hand is sufficient for stimulation of the FNR, and on the other hand, it allows the subject to obtain a sufficient amount of urine, since, with this type of study, diuresis in almost the majority of subjects is not less than 30-40%, most often - 50-70% of the initial fluid consumed, which is on average from 100 to 200 ml. This amount of urine is sufficient for functional irritation of the bladder and subsequent urination, which allows the bladder to be completely emptied without significant residual amounts of urine. In the case of a decrease in the load of less than 0.5% of body weight, the amount of urine obtained does not always make it possible to obtain the volume of urination during the hour required for the study. Further increase in

the volume of the load leads to additional effects on the cardiovascular system: changes in the volume of circulating blood, blood pressure, heart function, which distorts the results obtained; and the renal response is the result of both changes in the body's water-salt balance and the work of the cardiovascular system..

During the day (for example, from 7 am of one day to 7 am of the next day), the daily amount of urine, creatinine of daily urine (for further calculation of creatinine excretion during spontaneous diuresis) and protein excretion per day during spontaneous diuresis were determined. At 7 am of the day following the day of the study, the patient completely empties the bladder, after which he is asked to drink 0.5% sodium chloride solution at the rate of 0.5% of body weight. Then, for an hour, the patient was at rest in a supine position. After an hour, urine was collected, its amount was measured and urine protein, creatinine concentration were determined using the standard method; creatinine excretion, GFR after induced salt diuresis were calculated.

The studies were performed in compliance with the provisions of the Council of Europe Convention on Human Rights and Biomedicine and the recommendations of the Bioethics Committee under the President of the Academy of Medical Sciences of Ukraine.

The study materials were statistically processed using the BioStat program. The indicators identified during the study are presented as means ± standard deviation.

Results of the study and their discussion

The results obtained indicate that all examined patients were diagnosed with chronic kidney disease based on the presence of urinary syndrome for more than three months of observation. The most important indicator of urinary syndrome is proteinuria, which was observed in all patients with daily diuresis (Table 1). However, the patients did not show signs of chronic renal failure, judging by the fact that the level of creatinine in the blood plasma in all patients was within normal limits and on average amounted to 90.3±1.5 μmol/l. Kidney function after water-salt loading changed significantly: diuresis significantly increased almost threefold, mainly due to an increase in the glomerular filtration rate (Table 1). At the same time, water reabsorption almost did not change, as evidenced by the concentration of creatinine in the urine - 5.31±0.43 during daily and 5.71±9.91 mmol/l after water-salt loading.

In a portion of urine during spontaneous diuresis, the subjects excreted an average of 0.51 g/l of protein, and after salt-induced diuresis - 0.61 g/l, which is 10.1% more, respectively.

Proteinuria in a single portion of urine excreted by patients after salt loading, compared with spontaneous diuresis, increased in 11 out of 20 patients (55%), decreased in 8 patients (40%) and was the same in 1 subject (5%).

Table 1

Renal function in patients with proteinuria during daily induced water-salt diuresis (M±m)

Kidney function index	Number of people surveyed	Daily diuresis	Water-salt induced diuresis
Urination rate, ml/min	20	1,44±0,09	4,16±0,46 p<0,001
GFR by creatinine clearance, ml/min	20	88,5±5,88	232,5±31,62 p<0,01
Concentration, protein g/l	20	0,51±0,19	0,61±0,25
Protein excretion * 10-6μg/min	20	877,3±378,9	2045,8±790,46
Specific proteinuria, μg/1 mmol creatinine*10-3	20	107,9±44,17	167,6±82,82
Specific proteinuria, μg/1ml GFR*10-6	20	10,1±4,35	15,8±7,73

p – the reliability of differences between the groups under study.

Table 2

Specific proteinuria per 1 ml of glomerular filtrate **(**M±m)

With spontaneous diuresis x 10^{-6} mg	After saline induced diuresis x 10^{-6} mcg
2,33	1,49
3,26	2,23
0,67	1,25
0,32	0,39
3,25	4,35
0,59	0,83
2,49	2,6
0,44	0,44
0,14	1,62
1,12	1,43
68,9	143,2
1,64	3,1
2,33	2,93
0,48	1,38
4,4	5,87
0,72	1,04
0,86	1,52
25,1	39,6
54,6	66
28,5	34,4
$M_1 \pm м_1$ = 10,406 ± 4,45	$M_2 \pm м_2$ =16,837 ± 39,284
σ =19,438	σ = 175,59
C_v = 186,8 %	C_v = 1042,93 %

Daily protein excretion during spontaneous diuresis was on average 877.3 x 10-6 μg/min, and after salt loading – 2045.75 x 10-6 μg/min, which is 2.33 times higher (or 233.2%). Daily

protein excretion after salt loading, compared with spontaneous diuresis, increased in 19 subjects (95%) and decreased in 1 (5%).

Specific proteinuria was calculated by us per 1 mmol of creatinine and per 1 ml of glomerular filtrate [table 2]. Specific proteinuria per 1 mmol of creatinine during spontaneous diuresis was on average 107.88 x 10-3 mg, and after induced salt diuresis – 434.85 x 10-3 mg, which is 4.03 times higher, respectively. Specific proteinuria per 1 mmol of creatinine after induced saline diuresis compared to spontaneous diuresis increased in 16 subjects (80%), decreased in 6 subjects. (20 %).

Specific proteinuria per 1 ml of glomerular filtrate during spontaneous diuresis was on average 10.1 x 10-6 μg, after salt loading – 15.8 x 10-6 μg, which is 1.6 times higher (or 156.4%). Specific proteinuria per 1 ml of glomerular filtrate after induced salt diuresis compared to specific proteinuria during spontaneous diuresis increased in 17 subjects (85%), decreased in 2 subjects (10%) and was the same in 1 subject (5%).

Thus, specific proteinuria after salt loading compared to spontaneous diuresis in terms of 1 mmol of creatinine and 1 ml of glomerular filtrate increases in most subjects: by 1 mmol of creatinine - in 80% of subjects it increased, and by more than 15%, it increased, respectively, in 70% of subjects. Specific proteinuria after salt loading per 1 ml of CF increased in 85% of subjects from 4.4% to 1057% compared to spontaneous diuresis, i.e. hidden proteinuria occurs in 16 subjects (an increase of more than 15 %).

At the same time, protein excretion per day after salt loading decreased, and specific proteinuria after salt loading per 1 mmol of creatinine and 1 ml of KF increased in 1 subject, which confirms the value of this method for determining hidden proteinuria.

The fact that it is inappropriate to compare only proteinuria of urine portions during spontaneous and induced salt diuresis also indicates in favor of the method proposed by us, since in 6 of our subjects (30%) the protein of the urine portion after salt loading decreased, and specific proteinuria per 1 mmol of creatinine and per 1 ml of glomerular filtrate increased, i.e. hidden proteinuria occurred.

Thus, in patients with proteinuria without signs of chronic renal failure, water-salt loading is accompanied by an increase in the glomerular filtration rate, which indicates the presence of a functional renal reserve. At the same time, proteinuria increases, which indicates its dependence on the degree of filtration and, in turn, can contribute to additional damage to the nephrons [1]. In this case, it is necessary to determine the reasons for the increase in proteinuria. In our opinion, it is important to determine whether each functioning nephron loses more protein, because the load on it increases due to filtration and the “reabsorption threshold” is exceeded. The answer to this can be provided by calculating the specific proteinuria per unit of glomerular filtration or creatinine

excretion. This makes it possible to provide characteristics not only of total protein losses by the kidneys, but also of each functioning nephron, which expands their capabilities to determine the degree of their damage.

Conclusions

1. With water-salt loading, glomerular filtration rate, diuresis and total proteinuria increase.

2. Determination of latent proteinuria is a more informative indicator of kidney damage than comparing protein excretion during spontaneous, daily and induced water-salt diuresis.

3. The ratio of specific proteinuria to excretion of mmol creatinine or 1 ml of glomerular filtrate in % during salt loading compared to spontaneous daily diuresis is an important criterion for the degree of kidney damage.

4. Specific proteinuria is a marker of nephron damage, since it shows the true amount of protein that the nephron loses under conditions of its functional provocation; therefore, the study of specific proteinuria is quite relevant in the preclinical stages of kidney damage and can serve as one of the screening tests in risk groups with urinary system pathology.

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