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THROMBOMODULIN AND VON WILLEBRAND FACTOR AS MARKERS OF ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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The aim of research was to study the levels of thrombomodulin (TM) and von Willebrand factor (VWF) in the serum and urine of patients with chronic kidney disease (CKD)as diagnostic markers of endothelial dysfunction. The study involved 140 patients with CKD. The clinical diagnosis was determined based on standard methods of patients examination according to the kidney diseases classification and protocols of CKD patients management. The concentrations of TM and VWF in serum and urine were quantified by ELISA. A generalized endothelial dysfunction in the vessels of the whole body, including the kidneys and high concentration of TM and FVF in the serum and urine of patients with a diabetic nephropathy have been found. The concentration of TM and VWF in the serum of patients with a chronic glomerulonephritis was at the same level as in the serum of healthy individuals, while those in urine significantly exceeded the control values, indicating endothelial damage in the glomeruli of the kidneys due to exposure to pro-inflammatory cytokines. In our opinion, the studied markers will contribute to the timely diagnosis of endothelial dysfunction in patients with CKD and to the development of criteria for prescribing antiplatelet agents in glomerular kidney disease.

K e y w o r d s: thrombomodulin, Von Willebrand factor, endothelial dysfunction, chronic kidney disease, glomerulonephritis, diabetic nephropathy.

Chronic kidney disease (CKD) increases the length of hospital stay, impairs quality of life, requires lifelong monitoring and treatment [1]. The development of glomerulosclerosis is the basis for the formation of chronic renal failure, so the assessment of the degree of damage tothe glomerular apparatus of the kidneys is important for patients with glomerular diseases (glomerulonephritis (GN), diabetic nephropathy (DN), etc.) [2].

Some studies have shown that in the process of immune inflammation in the glomeruli of the kidney there is local activation of the hemostasis system associated with the stimulating effect of a number of cytokines produced by immune system cells on vascular hemostasis, plasma coagulation and fibrinolysis. Impaired blood coagulation leads to changes in microcirculation in the capillaries of the glomeruli, which impairs renal function, and the gradual transformation of fibrin into hyaline - to sclerosis of renal tissue [3].

In order to prevent the development of thrombosis, it is important to early diagnose conditions called thrombophilia. Thrombophilia is predisposition to clotting, or the risk of intravascular thrombus formation, it corresponds to the period when there is no intravascular coagulation, but there are already some changes in hemostasis, which in the future may cause its appearance. One such condition is endothelial dysfunction (ED) [4].

The kidneys have about 30% of the entire endothelial lining of the body's vessels (glomerular capillaries), and they have a large pool of endothelial cells, which is the first barrier to ultrafiltration in the glomerular capillaries [5]. Vascular endothelial cells normally have high antiplatelet anticoagulant and fibrinolytic activity. Decreased thromboresistance of the vascular wall makes a significant contribution to thrombogenesis [6].

Von Willebrand factor (VWF) is a high molecular weight polymer glycoprotein that is released into the bloodstream only by activated endothelium. It plays an important role as a mediator of platelet adhesion and aggregation. Currently, VWF is considered the gold standard in the assessment of ED

[7, 8]. Thrombomodulin (TM) is another molecule in this category. It acts as a cofactor of protein C activation and has anticoagulant activity. Soluble thrombomodulin can be secreted only from damaged endothelial cells, so it is considered a marker of endothelial damage [9, 10].

Detection of VWF and TM in high concentrations in the blood and urine may already serve as an indication for the use of antiplatelet agents, even in the absence of signs of thrombosis, in order to prevent it.

The aim of our research was to study the diagnostic markers of ED in the serum and urine of patients with CKD.

Materials and Methods

During the study, a standard examination was performed on all patients, which included general clinical, biochemical and instrumental research methods. Biochemical tests and enzyme-linked immunosorbent assays (ELISAs) were performed in the laboratory of Ivano-Frankivsk Regional Clinical Hospital. The research was performed in accordance with international standards for the coordinated participation of respondents, the ethical component of research and biomaterial collection (protocol of ethical commission No 96/18 from 29.11/2018). All patients signed a written informed consent to participate in the study.

We conducted a prospective study involving 140 patients with CKD who were hospitalized at the Ivano-Frankivsk Regional Clinical Hospital (Ukraine) during 2018-2021. Of these patients, 100 patients (71.4%; 95% CI 53.4-76.7) had glomerulonephritis (GN) and 40 patients (28.6%; 95% CI 21.3-36.8) had diabetic nephropathy (DN). The average age of the patients was 46 years (41; 49). Among the patients, there were more men (n = 92, 65.7%; 95% CI 57.2-73.5) than women (n = 48, 34.3%; 95% CI 26.5-42.8).

CKD stage I was diagnosed in 36 patients (25.7%; 95% CI 18.7-33.8), CKD stage II – in 21 patients (15.0%; 95% CI 9.5-22.0), CKD stage IIIa – in 24 patients (17.2%; 95% CI 11,3-24,4), CKD stage IIIb – in 31 patients (22.1%; 95% CI 15.6-29.9) and CKD stage IV – in 28 patients (20.0%; 95% CI 13.7-27.6).

In 86 patients (61.4%; 95% CI 52.8-69.5) with GN and DN, urinary syndrome was present, and in 54 patients (38.6%; 95% CI 30.5-47.2), nephrotic syndrome occurred.

In 25 patients (17.9%; 95% CI 11.9-25.2), the diagnosis of GN was confirmed morphologically, as follows: in 11 patients (44.0%; 95% CI 24.4-65.1), mesangioproliferative GN was confirmed, and 5 patients (20.0%; 95% CI 6.8-40.7) had membranous nephropathy, 4 patients (16.0%; 95% CI 4.5-36.1) had focal-segmental glomerulosclerosis, 3 patients (12.0%; 95% CI 2.5-31.2) had nephropathy with minimal changes, and 2 patients (8.0%; 95% CI 1.0-26.0) had membrane proliferative (mesangiocapillary) GN.

Also, 40 almost healthy individuals were selected who formed a comparison group and were representative of the main group.

The clinical diagnosis was determined based on standard methods of examination of patients according to the classification of kidney diseases and protocols of management of patients with CKD.

The glomerular filtration rate (GFR) was determined using a CKD-EPI calculator (https://nephrology.kiev.ua/eGFR/gfr.htm). Daily protein excretion (DPE) in urine collected within 24 h was determined by colorimetric method (Dialab, Wiener Neudorf, Austria). Urine was stored at a temperature of 2-25°C. DPE reference value: <300 mg/day [11].

Concentration of TM and VWF were quantified in serum and urine by using a set of reagents Human TM Elisa Kit (Elabscience, USA) and Human VWF Elisa Kit (Elabscience (USA)). Blood obtained from the patient's ulnar vein (on an empty stomach) was used to study concentration of TM and VWF. Blood serum was separated from erythrocytes by centrifugation (3000 rpm) for 10 min. Supernatants were stored at - 12°C until use. For the study, a morning portion of urine was collected, centrifuged (1500 rpm) for 10 min and 1-2 ml of supernatant were used to determine thrombin. Supernatants were stored at - 12°C until use. Detection Range VWF concentrations: 1.56-100 ng/ml. Sensitivity: 0.94 ng/ml. Detection Range TM concentrations: 62.50-4000 pg/ml. Sensitivity: 37.50 pg/ml [7, 12].

STATISTICA 8 software (StatSoft, Serial STA862D175437Q) was used for statistical analysis. The frequency of qualitative indicators was presented in absolute (n) and relative (%) frequencies with the indication of the 95% confidence interval (CI) in the form of "n (%; 95% CI)". When analyzing quantitative data, it was necessary to determine the nature of the distribution of indicator values using Shapiro-Wilk's test. For quantitative data with a normal distribution, the results were represented as "M (σ)," where M is the mean value and σ is the standard

deviation. For quantitative data with an abnormal distribution, "Me (q_1; q_2)" was used, where Me is the median and q_1; q_2 are quartiles. Quantitative indicators with normal distribution of values in 2 independent groups were compared using the Student's criterion. Quantitative parameters with abnormal distribution in 2 independent groups were compared using the Mann-Whitney method. A comparison of 2 independent groups for the qualitative indicator was carried out according to the exact Fisher criterion.

The correlation of normal distributions was assessed by determining the Pearson correlation coefficient, and in the abnormal distribution by the Spearman's rank correlation coefficient. A *P*-value of < 0.05 was considered to be statistically significant.

Results and Discussion

As can be seen from Table 1 we noted significantly higher concentration of TM and VWF in the serum of patients with CKD: DN compared with the group of almost healthy individuals and the group of patients with GN ($P < 0.05$; $P_1 < 0.05$), and concentration of these markers in the urine was significantly higher than values of the norm group ($P < 0.05$), which is explained by the fact that patients with diabetes have generalized ED in the vessels of the whole organism, including the vessels of the kidneys.

In patients with CKD: GN, concentration of TM and VWF in the serum did not differ significantly from the normal group ($P > 0.05$), but their level in the urine significantly exceeded the value in the group of healthy individuals ($P < 0.05$) , which may indicate endothelial damage in the glomeruli of the kidneys, due to exposure to pro-inflammatory cytokines.

The presence of TM and VWF in the urine of patients with GN and DN indicates the possibility that some of them are excreted in the urine when the endothelium in the affected glomeruli of the kidneys is damaged.

When we analyzed the results of the study in all patients with CKD, we found a medium-strength inverse correlation between GFR and concentration of TM ($r = -0.56$; $P < 0.05$) in serum and GFR and concentration of VWF ($r = -0.48$; $P < 0.05$) in serum, indicating the progression of ED with decreased renal function (Fig. 1).

We also noted a direct medium-strength correlation between DPE and concentration of TM in urine ($r = 0.63$; $P < 0.05$) and DPE and concentration of VWF ($r = 0.54$; $P < 0.05$) in urine in patients with CKD: GN, which indicates that the concentration of TM and VWF in the urine reflects the degree of inflammation in the kidneys, and their determination can be used to assess the activity of GN (Fig. 2).

Most of the studies available in the literature are devoted to the study of the concentrations of TM and VWF in the serum of patients with CKD and confirm the occurrence of ED with reduced

Table 1. Concentration of thrombomodulin and von Willebrand factor in the serum and urine of patients with chronic kidney disease and healthy individuals

Indicator (concentration)	Healthy individuals ($n = 40$)	Patients with chronic kidney disease ($n = 140$)	
		With glomerulonephritis ($n = 100$)	With diabetic nephropathy ($n = 40$)
Thrombomodulin in serum, pg/ml Me (q ; q_2)	74.8 (63.6; 89.7)	97.5 (75.8; 105.4)	464.3 (312.9; 608.7) $P < 0.05$, $P_1 < 0.05$
Thrombomodulin in urine, pg/ml Me (q_1; q_2)	32.4 (24.7; 46.5)	213.6 (178.7; 267.9) $P < 0.05$	245.3 (196.7; 291.4) $P < 0.05$
Von Willebrand factor in serum, ng/ml Me (q_1; q_2)	26.4 (18.6; 39.5)	32.2 (24.6; 41.7)	87.1 (68.6; 95.8) $P < 0.05$, $P_1 < 0.05$
Von Willebrand factor in urine, ng/ml Me (q_1; q_2)	8.4 (5.5; 10.1)	59.7 (44.8; 72.9) $P < 0.05$	62.5 (49.8; 81.7) $P < 0.05$

Note: *P* – the reliability of the difference in patients with chronic kidney disease: glomerulonephritis and diabetic nephropathy in comparison with a group of almost healthy individuals; P_1 – the reliability of the difference in the indicators of patients with diabetic nephropathy in comparison with the group of patients with glomerulonephritis; Me (q_1; q_2) is the median and quartile

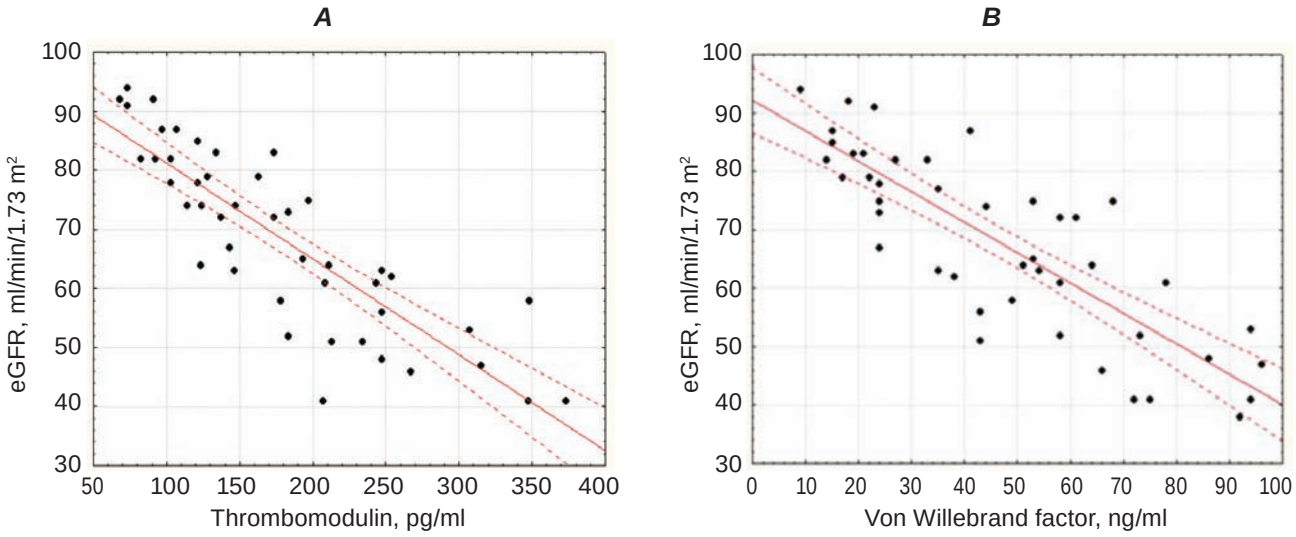

*Fig. 1. Correlation between concentrations of thrombomodulin (**A**) and von Willebrand factor (**B**) in serum and glomerular filtration rate in patients with chronic kidney disease*

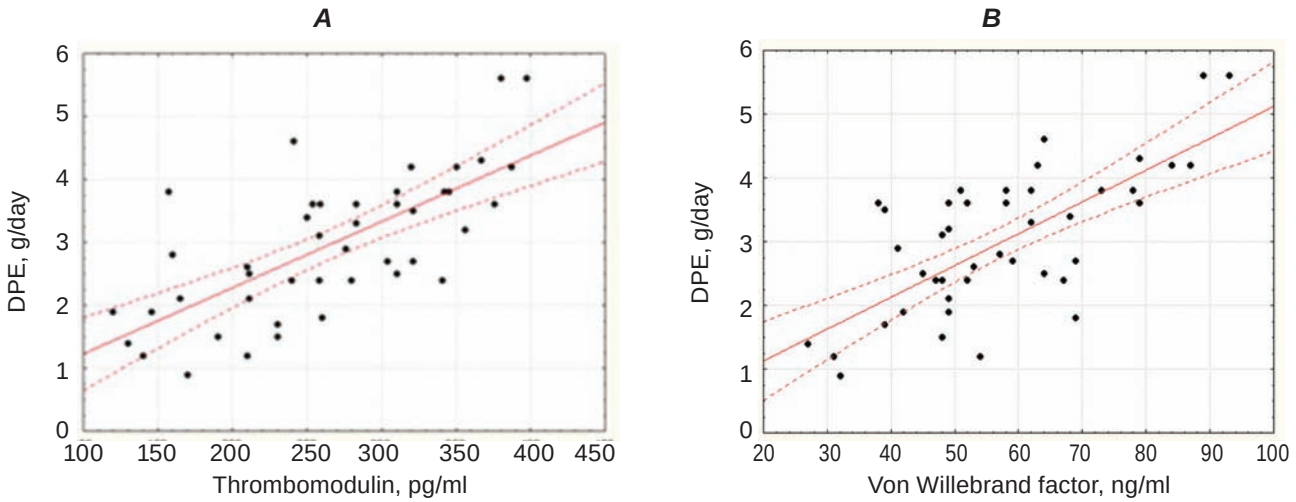

*Fig. 2. Correlation between concentrations of thrombomodulin (**A**) and von Willebrand factor (**B**)in urine and daily protein excretion in patients with chronic kidney disease: glomerulonephritis*

GFR [13]. However, there are isolated studies that confirm the opinion that a number of prothrombotic biomarkers in urine are markers of renal disease activity and precursors of GFR reduction in CKD [14].

Several experimental studies have shown that in membranoproliferative GN and lupus GN the amount of TM expressed by glomerular endothelial cells increases, and this finding is a marker of disease activity. Also, researchers note that the value of increased expression of endothelial anticoagulant glycoprotein in diseases characterized by pathological intraglomerular coagulation is unknown and requires further research [10, 15].

High concentrations of VWF in the blood and urine of patients with CKD may be an indication for the use of antiplatelet therapy in these patients, as VWF is a major mediator of platelet activation and adhesion, which promotes the activation of the vascular-platelet link of hemostasis [8].

Determination of TM in blood and urine may be an early marker for the diagnosis of ED, because soluble thrombomodulin can be secreted only from damaged endothelial cells [9].

In this way, in diabetes, there is generalized endothelial dysfunction in the vessels of the whole organism, including in vessels of kidneys that is

confirmed by high concentrations of thrombomodulin and Von Willebrand factor both in serum and in urine of patients with CKD: DN. In patients with CKD: GN, there is damage to the endothelium in the glomeruli of the kidneys, due to exposure to proinflammatory cytokines, as evidenced by high concentrations of TM and VWF in the urine, at normal values of these biomarkers in the serum. Determination of concentrations of this markers in urine as markers of endothelial dysfunction in the glomeruli of the kidney will allow timely use of antiplatelet therapy and evaluate the effectiveness of this therapy, which can significantly change the course of CKD, serve as early prevention of thrombosis and cardiovascular events in patients with CKD.

Prospects for further research. In our opinion, it would be interesting to study the levels of TM and VWF depending on the treatment received (cytostatics, hormones, anticoagulants, antiplatelet agents), as well as depending on the morphological variant of GN.

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ТРОМБОМОДУЛІН І ФАКТОР ВІЛЛЕБРАНДА ЯК МАРКЕРИ ЕНДОТЕЛІАЛЬНОЇ ДИСФУНКЦІЇ У ПАЦІЄНТІВ ІЗ ХРОНІЧНОЮ ХВОРОБОЮ НИРОК

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Метою дослідження було оцінити рівень тромбомодуліну (ТМ) та фактора Віллебранда (ФВ) як діагностичних маркерів ендотеліальної дисфункції у сироватці крові та в сечі пацієнтів із хронічною хворобою нирок (ХХН). До дослідження було залучено 140 пацієнтів із ХХН. Клінічний діагноз визначали на основі стандартних методів обстеження пацієнтів відповідно до класифікації захворювань нирок та протоколів ведення пацієнтів із ХХН. Концентрацію ТМ та ФВ визначали у сироватці крові та сечі методом ELISA. У хворих на діабетичну нефропатію виявлено генералізовану ендотеліальну дисфункцію у судинах усього організму, у тому числі і в судинах нирок та високу концентрацію ТМ і ФВ у сироватці та сечі. Концентрація ТМ та ФВ у сироватці крові хворих на хронічний гломерулонефрит та у здорових осіб була однаковою, тоді як у сечі пацієнтів із ХХН вона перевищувала контрольні значення, що свідчило про ураження ендотелію клубочків нирок через вплив прозапальних цитокінів. На нашу думку, досліджені маркери можуть сприяти вчасній діагностиці ендотеліальної дисфункції у пацієнтів із ХХН та допоможуть розробці критеріїв у призначенні антитромбоцитарних засобів за гломерулярних уражень нирок.

Ключові слова: тромбомодулін, фактор Віллебранда, ендотеліальна дисфункція, хронічна хвороба нирок, гломерулонефрит, діабетична нефропатія.

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