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Research Article

Albuminuria as an Independent Factor of Cardiovascular Complications in Patients with Comorbid Pathology

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Abstract

The necessity of determining and assessing the dynamics of albuminuria and glomerular filtration rate in patients with type 1 and type 2 DM (as an early marker of endothelial dysfunction and a predictor of cardiovascular complications) was substantiated amid pathogenetic therapy with glycosaminoglycan sulodexide. There is a clinically significant decrease in the levels of both total protein and albumin in the urine, which along with an increase in glomerular filtration rate indicates an improvement in renal microcirculation. It is worth pointing out that the increase in glomerular filtration rate is mostly evident among type 2 DM patients with comorbid non-alcoholic fatty liver disease.

Keywords: Diabetes Mellitus; Albuminuria; Endothelial Dysfunction; Non-alcoholic Fatty Liver Disease; Treatment

Abbreviations

AH: Arterial Hypertension; GAGs: Glycosaminoglycans; LVH: Left Ventricular Hypertrophy; DN: Diabetic Nephropathy; MI: Myocardial Infarction; IR: Insulin Resistance; AC: Atherogenic Coefficient; NAFLD: Non-alcoholic Fatty Liver Disease; TC: Total Cholesterol; DM: Diabetes Mellitus; GFR: Glomerular Filtration Rate; CVD: Cardiovascular Disease; TG: Triglycerides; CKD: Chronic Kidney Disease; HDL: High-density Lipoprotein; LDL: Low-density Lipoprotein; ED: Endothelial Dysfunction

Cardiovascular disease (CVD) is a leading cause of morbidity, disability, and mortality in patients worldwide. The incidence of DM increases cardiovascular risk compared with myocardial infarction (MI) [1,2]. In addition, CVD is by far the most frequent cause of death in chronic kidney disease (CKD), and the latter, in turn, represents an independent risk factor for cardiovascular pathology and mortality [3,4].

Introduction : The concept of combining cardiac and renal pathology and distinguishing different types of the cardiorenal syndrome was described in 2008. Meanwhile, it should be noted that the development of cardiorenal disorders has long been a matter of concern for scientists and clinicians. Back in 1836, R. Bright pointed out the probable relation of arterial hypertension (AH) and left ventricular hypertrophy (LVH) development to nephritic renal damage, and E.M. Tareev considered the issue of renal dysfunction in the genesis of AH, LVH, and the development of congestive kidney in heart failure in his monographs "Hypertension" (1948) and "Nephritis" (1958).

DM is a major cause of abnormal protein excretion in the urine. Especially, when it is combined with AH. These pathological conditions are particularly dangerous for kidney damage as target organs. The AH is associated with obesity, type 2 DM, insulin resistance (IR), hyperlipidemia, and atherosclerosis. Therefore,

high cardiovascular risk comes as a consequence. These diseases share common pathogenetic mechanisms that define their development and progression and can potentiate and aggravate the development of each other [5]. The incidence of cardiovascular, cerebrovascular, and peripheral vascular diseases, as well as the occurrence of nephropathy and retinopathy, has been proved to be significantly greater in patients with type 2 DM [6].

In 1999, the World Health Organization marked microalbuminuria as one of the components of metabolic syndrome, which indicates a considerable negative contribution of this factor to cardiovascular morbidity and mortality in AH patients with concomitant DM. A little later, WHO experts advised determining annual albumin excretion in urine in type 1 DM patients over 12-15 years old 5 years after the disease onset and in all type 2 DM patients under 70 years old [7,8].

The term "microalbuminuria" previously used by US experts has been replaced by "moderately increased albuminuria". The latter is the crucial early sign of renal damage, reflecting the initial stages of vascular pathology (endothelial dysfunction (ED), atherosclerosis), which consistently correlates favorably with an increase in CVD and mortality. When analyzing the results of clinical trials, even the lowest levels of increased albumin excretion in urine are directly associated with a significant rise in the risk of cardiovascular accidents, including fatal ones, and the progressive increase in albuminuria over time indicates deterioration of vascular health and, therefore, leads to an increased risk. It is worth emphasizing that the most widespread causes of progressive renal dysfunction are AH and DM, which are accounted for about two-thirds of the new cases of end-stage renal disease. According to the results of an empirical study with over 39,000 participants, the prevalence of albuminuria in DM was about 33%, increasing with concomitant AH to 35%, and in the presence of macro- and/or microvascular complications to 38-39% [10,11]. The risk of albuminuria was found to increase with the disease course. The UKPDS study showed that albuminuria was diagnosed in 12% of patients with newly diagnosed type 2 DM and almost 30% of patients with a disease course exceeding 12 years. A rough estimate of the new cases of albuminuria in patients with diabetes ranges from 1% to 3% per year [12].

Moderately increased albuminuria is common not only in diabetic nephropathy, but it is a sensitive and early precursor of cardiovascular risk in patients with AH, regardless of DM or in combination with existing renal pathology. Moderately increased albuminuria is proved to be an independent factor of cardiovascular risk and the earliest preclinical sign of involvement of vulnerable target organs, such as kidneys. Moreover, albuminuria is associated with several additional risk factors such as obesity, smoking, IR, LVH, LV dysfunction, increased c-reactive protein levels, and dyslipidemia. Several studies indicate a high frequency of albuminuria in obese people [13]. Thus, the prevalence of albuminuria in obese individuals (in the absence of DM and renal pathology) was studied. The results showed that the daily albumin excretion in these patients was, on average, much higher than in those with normal body weight. In obese patients, albuminuria occurred in 12.1% of cases, and when obesity was combined with AH, its prevalence increased to 19.2%. When various risk factors are combined, especially with AH and/or DM, the probability of abnormal renal function increases significantly.

Currently, there is a sufficient evidence base, confirming that timely diagnosis of kidney involvement in AH and/or DM and the adoption of nephroprotective approaches when treating such patients improves prognosis, reduces CVD and mortality, and delays the development of irreversible changes in renal tissue and subsequent renal impairment. Thus, a 10-year prospective study showed that the occurrence of microalbuminuria in AH patients was a precursor of coronary heart disease [14]. One LIFE sub-study (K. Wachtell., *et al*. 2002) found that increased protein excretion in urine was clearly associated with LVH (confirmed by electrocardiography), regardless of age, gender, race, BP level, DM, smoking habits, and serum creatinine level [15]. Similar data were obtained in another LIFE sub-study (M.H. Olsen., *et al*. 2004), where LVH was assessed by echocardiography. According to this study, increased albumin/creatinine ratio correlated with increased incidence of cardiovascular accidents (cardiovascular death, non-fatal stroke, MI) [16].

The HOPE study reported that increasing the albumin/creatinine ratio in the urine by every 0.4 mg/mmol above standard would increase the risk of severe cardiovascular accidents by 5.9% [17].

Furthermore, the DIABHYCAR study (the type 2 DIABetes, HYpertension, microalbuminuria or proteinuria, Cardiovascular events and Ramipril study, 2003) conclusively showed that albuminuria could increase the risk of heart failure [18].

The prognostic significance of moderately increased albuminuria to determine cardiovascular risk was further confirmed by the results of another large population study [19]. The peculiarity of this study lies in the fact that mainly unaffected individuals took part therein. Based on this study, one can see the validity of screening for albuminuria: the detection of increased protein excretion in the urine of a healthy person makes it possible to determine the early stages of vascular damage by atherosclerotic process and to identify high-risk groups [20]. Similar results were obtained in a large-scale prospective EPIC-Norfolk study (European Prospective Investigation into Cancer in Norfolk study, 2004), involving over 23,000 patients. According to this study, the presence of albuminuria indicated an increased risk of stroke [21].

A close correlation between albuminuria and cardiovascular morbidity was found even with very low protein excretion rates in the urine. For example, the Copenhagen City Heart-3 study found an increased risk of coronary heart disease and cardiovascular death (regardless of AH, DM, and renal disease) just at albuminuria levels >4.8 μg/min, which is significantly lower than the commonly used lower threshold for diagnosing albuminuria (20 μg/min) [22].

Volpe M. gave other evidence, which also pointed to the exceptional importance of albuminuria as an independent risk factor for CVD and mortality [11]. The decisive place of endothelial dysfunction (ED) in CVD progression is now evident. Endothelial dysfunction is the initial link of the cardiovascular continuum, leading the patient from the initial manifestations of the disease to death. Currently, endothelial dysfunction is considered the main mechanism of AH onset and progression, which has been conclusively confirmed by appropriate methods of peripheral, coronary, and renal arterial studies. Thus, the association of ED with renal damage in DM and AH seems to be logical, but the details of this correlation require further study.

The ED results in the imbalance of oppositely acting mediators [23], leading to vasospasm, proliferation of smooth muscle cells, platelet aggregation, leukocyte adhesion, and impaired angiogenesis. These changes are particularly expressed by muscle-type small arteries with a diameter of 100-300 μm, which are responsible for systemic vascular resistance [24]. Oxidative stress [25-27], which stands for the production of strong vasoconstrictors (endoperoxide, endothelin, angiotensin II), as well as cytokines and tumor necrosis factor that suppress NO production, are of primary importance in the pathogenesis of endothelial dysfunction [28,29].

The analysis of the reference sources suggests that albuminuria is one of the most reliable and accurate markers of endotheliocyte dysfunction, allowing to predict a high probability for both the deterioration of renal function and the development of cardiovascular complications [30,31]. Albuminuria is a consequence of increased albumin loss from plasma through the endothelium and is therefore defined as a diagnostic marker of systemic ED. The latter is confirmed by the fact that the presence of albuminuria, as a rule, correlates with the occurrence of ED signs during endothelium-dependent dilatation test in the brachial artery (HOORN, 2004). The ED is typical for the early stages of atherosclerosis development and is directly associated with increased cardiovascular risk [32].

The decisive factors in halting the progression of renal tissue changes include correction of arterial hypertension, carbohydrate metabolic compensation, and normalization of blood lipid profile. Meanwhile, one of the key areas of nephroprotective therapy is the use of drugs of the glycosaminoglycan (GAG) group, which have a high tropism to the vascular wall and are included in the standards of diabetic nephropathy (DN) therapy [33,34]. Currently, one of the most effective pathogenetic methods of ED treatment is considered to be the administration of sulodexide GAGs. Sulodexide contains two GAGs - fast-acting heparin fraction and dermatan sulfate, which restore the function and thromboresistance potential of endothelium and microvascular walls, having an antithrombotic effect. The advantage of sulodexide compared with conventional heparin and low molecular heparins lies in its effectiveness not only when administered parenterally, but also when taken orally [35-37].

This study was aimed at determining the dynamics of albuminuria and glomerular filtration rate (GFR) in patients with type 1 and type 2 DM and AH during GAG treatment with sulodexide.

Materials and Methods

40 patients were examined, including 20 patients with AH combined with type 1 DM (group 1) and 20 patients with type 2 DM and AH (group 2). The mean duration of DM was (12.3 ± 2.6) years and the mean patient age was (46.2 ± 1.3) years.

Patients were examined clinically; BMI, body weight, and height were calculated. The degree of carbohydrate metabolic compensation was assessed according to the WHO criteria (2006).

The clinical and biochemical analysis included determination of daily glycemic indexes by glucose oxidase method using Biosen C line express analyzer and Exan-G glucose analyzer, calculation of the average daily glycemic index, glycemic amplitude, and glycosylated hemoglobin (HbA1c) by colorimetric method.

Determination of the blood lipid profile included a study of total cholesterol (TC) levels using a set of LLC NPP Filisit-Diagnostica (Ukraine), high-density lipoprotein (HDL), calculation of low-density lipoprotein (LDL), and atherogenicity coefficient (AC) were performed according to generally accepted formulas. Triglyceride (TG) level was determined by enzymatic method; creatinine, blood, and urine urea were determined using a set of LLC NPP Filisit-Diagnostica; Reberg test was performed. Fibrinogen in plasma was determined by gravimetric analysis (normal values are 200-400 mg%); fibrinolytic activity was determined by plasma euglobulin lysis (normal values are 180-270 min); prothrombin (thromboplastin) time was determined (prothrombin activity of unaffected blood is 93-107%).

Albumin excretion in the urine was determined by enzyme immunoassay. Normal values of albumin in daily urine did not exceed 30 mg/day, and those in morning urine did not exceed 20 μg/ml.

The creatinine level in blood was used to calculate GFR using the CKD-EPI equation [38]. A computer program was used for convenient calculation.

The presence of albumin excretion in the urine or a decrease in GFR of less than 60 ml/min/1.73 sq m, as well as a combination of these processes, were considered for the DN diagnosis. As previously noted, the 2014 American Diabetes Association (ADA) guidelines suggested replacing the outdated terms “microalbuminuria” and “macroalbuminuria” with persistent albuminuria with albumin excretion of 30-299 mg/day and over 300 mg/day, given the continuous nature of the pathological process. Decrease in GFR to the level < 60 ml/min/1.73 sq m, persisting for 3 months or more, regardless of the level of albuminuria, indicates the presence of CKD, with the degree of decline in GFR determining its stage.

Along with type 1 or type 2 DM, all patients were diagnosed with diabetic nephropathy, CKD in the stage of persistent albuminuria.

In addition to antihypertensive drugs, consisting of inhibitors, oral antidiabetic drugs, and insulin therapy, sartans in standard dosages were also added to the patient’s therapy. All patients were also prescribed sulodexide intramuscularly at 600 IU a day for 10 days, followed by oral administration at 1000 IU a day for 60 days.

The results were statistically processed by calculating the arithmetic mean and the standard deviation. The reliability of differences was determined by Student’s t-test. Differences were considered statistically significant at $p < 0.05$. All calculations were performed on a Pentium PC in Windows XP using Excel XP, STATISTICA-6.0 software.

Results and Discussion

It should be noted that normal BP was achieved; carbohydrate metabolic subcompensation was observed in the examined patients, which was achieved by adequate antidiabetic therapy.

All the monitored patients initially had increased urinary excretion rates of both total protein and albumin, on average for the groups. Proteinuria and albuminuria were more evident in patients with type 1 DM. The presence of albuminuria can be regarded as a manifestation of generalized vascular endothelial dysfunction manifested as damage to glycocalyx, which lines the luminal surface of blood vessels and represents a hydrated structure consisting of proteoglycans, glycoproteins, and glycolipids associated with the endothelial cell membrane. Table 1 shows that the therapy produces a clinically significant decrease in the levels of both total protein and albumin in the urine, which along with an increase in GFR indicates an improvement in renal microcirculation. The increase in GFR was most pronounced in type 2 DM patients.

Value	Group 1, n = 20		Group 2, n = 20	
	Before treatment	After treatment	Before treatment	After treatment
Albumin in urine, mg/day	85.6 ± 12.3	51.25 ± 8.6 p < 0.05	45.15+6.4	27.13+5.2 p < 0.05
Protein in urine, g/l	0.46 ± 0.1	0.3 ± 0.08	0.32 ± 0.06	0.12 ± 0.05 p < 0.05

Serum creatinine, μmol/l	116.32 ± 5.92	111.55 ± 5.89	110.23 ± 5.23	102.04 ± 4.68
Creatinine in urine, mmol/day	10.86 ± 0.95	11.08 ± 1.3	10.91 ± 0.86	11.02 ± 1.2
Blood urea, mmol/l	6.36 ± 0.91	6.3 ± 0.85	6.7 ± 0.67	6.5 ± 0.82
Urea in urine, mmol/day	434.96 ± 24.14	414.27 ± 44.14	428.84 ± 28.17	408.16 ± 32.16
GFR (CKD-EPI), ml/min/1.72 sq m	57.56 ± 3.37	62.23 ± 5.97	60.15 ± 4.48	74.45 ± 5.23 $p < 0.05$
Tubular reabsorption, %	97.46 ± 0.27	96.83 ± 0.83	97.87 ± 0.43	97.23 ± 0.23

Table 1: Dynamics of protein excretion indexes, GFR, and certain values of renal function test in the monitored patients.

Note: p - reliability of differences between the index before and after treatment according to Student's t-test.

Given the features of the therapy, namely the administration of sulodexide, containing fast-acting heparin fraction and dermatan sulfate with an antithrombotic effect, the study of some indicators of patients' coagulogram was of particular interest. Manifestation of DN in DM patients is often accompanied by diabetic retinopathy in the proliferative stage, often with the development of hemorrhagic foci on the fundus. One should bear in mind the restriction of this drug due to the risk of exacerbation of retinal vessels: occurrence of hemorrhages, hemophthalmos, and retinal detachment in DM patients. Even though this study was not aimed at determining the degree of diabetic retinopathy and ocular fundus condition, when prescribing pathogenetic therapy with sulodexide in DM patients, it was of interest to study some parameters of coagulation and anti-coagulation system. The data obtained are presented in table 2.

Value	Group 1		Group 2	
	Before treatment	After treatment	Before treatment	After treatment
APTT, sec	27.88 ± 0.74	29.6 ± 0.76	27.67 ± 0.74	29.34 ± 0.76
Prothrombin index, %	96.58 ± 0.86	95.15 ± 1.3	96.49 ± 0.76	95.17 ± 1.2
Fibrinogen concentration, g/l	2.92 ± 0.21	2.91 ± 0.23	2.96 ± 0.27	2.91 ± 0.19
Fibrin, mg	13.17 ± 0.96	13.06 ± 1.04	13.15 ± 0.86	13.05 ± 1.12
Fibrinolytic activity, min	268.1 ± 7.1	237.78 ± 14.3	269.1 ± 8.4	240.62 ± 13.2

Table 2: Dynamics of coagulogram indices in the monitored patients.

Table 2 shows that there are no clinically significant changes in coagulogram indices on average for the groups. Thus, we can assume that the administration of sulodexide does not contribute to the development of hemorrhagic complications and aggravation of diabetic retinopathy in type 1 and type 2 DM patients.

Due to the DM epidemic and, consequently, the progressive growth of NAFLD, the need for early detection of renal pathology in this category of patients to stratify the risk of cardiac events is crucial. Oxidative stress, impaired proteoglycan synthesis (heparan sulfate and dermatan sulfate), impaired cytokine and growth factors production, as well as vasoactive factors (angiotensin II, endothelin) mostly expressed in DM combined with NAFLD contribute to increased intrarenal pressure, development and progression of CKD. In addition, the presence of NAFLD in DM patients contributes to severe dyslipidemia, which aggravates the course of DN in these patients.

According to the hypothesis of "nephrotoxic" lipid action, dyslipidemia results in glomerular capillary endothelium damage,

promotes the transformation of mesangiocytes into xanthome cells, stimulates mesangiocyte proliferation, and increases extracellular matrix component production by glomerular and tubule cells. Meanwhile, renal pathology contributes to the development and aggravation of dyslipidemia due to increased synthesis of LDL and loss of lipoprotein-decomposing enzymes in urine. Thus, it is of interest to study the dynamics of cholesterol levels and their fractions in DM patients. The obtained data are presented in table 3.

Value	Group 1		Group 2	
	Before treatment	After treatment	Before treatment	After treatment
TC, mmol/l	5.36 ± 0.5	5.93 ± 1.5	6.04 ± 0.68	5.46 ± 2.3
HDL, mmol/l	1.22 ± 0.05	1.27 ± 0.08	1.23 ± 0.1	1.17 ± 0.12
AC	3.44 ± 0.53	3.8 ± 1.46	3.86 ± 0.64	3.68 ± 2.16
TG, mmol/l	3.57 ± 1.86	2.43 ± 1.5	4.08 ± 1.6	2.76 ± 1.8
LDL, mmol/l	2.98 ± 0.5	2.3 ± 0.7	3.18 ± 0.9	2.6 ± 0.6
VLDL, mmol/l	1.6 ± 0.84	1.08 ± 0.65	1.5 ± 0.72	1.18 ± 0.67

Table 3: Dynamics of lipid metabolism indices in the monitored patients.

Table 3 shows that there are no clinically significant changes in lipidogram indices in both DM-type patients during the therapy. Target values of blood lipid spectrum are not achieved, which can be explained by both the lack of a significant effect of the therapy on cholesterol and its fractions, and the excessive synthesis of LDL in diabetic nephropathy, which levels the positive effects of therapy on lipid metabolism in DM and CKD patients.

Data on modern methods of diagnosing and treating DM and its complications are essential for a general practitioner. Since DN can develop asymptomatically for several years, its manifestations, such as general weakness, decreased appetite, edema, and headaches, are typical only for the clinically evident stage. The first preclinical DN sign is persistent albuminuria. Considering albuminuria as a precondition for cardiovascular accidents, it makes sense to conduct regular urinary protein excretion tests not only for DM patients but also for the broader population. Quantitative detection of urinary albumin not only reveals the risks, but also its extent, as well as assesses the dynamics of the progression of renal impairment and, consequently, the increase in cardiovascular risk. GFR calculation and albuminuria estimation are available markers of target organ damage with high prognostic values.

We believe that studying possible methods of nephroprotection, which is the most effective at the stage of functional changes (without organic lesion) and capable both of slowing down and halting the pathological process in kidneys or even contributing to its reverse development, is of particular importance for clinical practice.

There is no doubt that optimal glycemic control is the main approach to preventing or slowing down the progression of types 1 and 2 DM. Meanwhile, an important component of DN pathogenetic therapy is the correction of endothelial dysfunction and structural changes in glycocalyx and basal membranes of renal glomeruli.

Conclusions

- The necessity of determining and assessing the dynamics of albuminuria and glomerular filtration rate in patients with type 1 and type 2 DM (as an early marker of endothelial dysfunction and a predictor of cardiovascular complications) was substantiated.
- Following the glycosaminoglycan sulodexide therapy, there is a clinically significant decrease in the levels of both total protein and albumin in the urine, which along with an increase in glomerular filtration rate indicates an improvement in renal microcirculation. It is worth pointing out that the increase in glomerular filtration rate is most evident among type 2 DM patients with comorbid non-alcoholic fatty liver disease.

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