SCIREA Journal of Clinical Medicine
ISSN: 2706-8870
http://www.scirea.org/journal/CM
August 31, 2024
Volume 9, Issue 3, June 2024
https://doi.org/10.54647/cm321287

The role of adhesion molecule expression in the development of morphological changes in renal tissue in patients with type 2 diabetes

T.S. Ryabova[1] , I.A. Rakityanskaya[2]

[1] S.M. Kirov Military Medical Academy, Department of Nephrology and Efferent Therapy, 194044, Saint Petersburg, st. Academician Lebedeva, 6, Russia

[2] Department of Allergology, Immunology and Clinical Transfusiology, Municipal Outpatient Hospital no. 112, 195427, Saint Petersburg, st. Academician Baykova, 25/1, Russia

Tatiana S. Ryabova doctor of medical sciences, assistant professor

ORCID: https://orcid.org/0000-0001-9543-9646

Irina A. Rakityanskaya doctor of medical sciences, professor

ORCID: https://orcid.org/0000-0003-2524-4602

Abstract

Research objective. The work is devoted to studying the role of the expression of intercellular adhesion molecules-1 (ICAM-1, CD54), P-selectin (CD62P) and E-selectin (CD62E) in renal tissue in the development and progression of morphological changes in diabetic nephropathy in patients with type 2 diabetes mellitus.

Material and methods. The examination was carried out in 50 patients with type 2 diabetes mellitus (T2DM) (average age 66.58±3.27 years). The duration of diabetes was 14.744±0.1.062 years. All patients underwent intravital puncture biopsy of the kidney. To

confirm the morphological diagnosis, light and immunofluorescence microscopy of kidney tissue biopsies were performed. Morphological changes in tissue were assessed in accordance with the latest international classification of diabetic nephropathy, developed in 2010. According to light microscopy, class IIa (mild mesangial expansion) was identified in 12 patients, class IIb (severe mesangial expansion) was identified in 14 patients, in 19 patients – class III (nodular Kimmelstiel-Wilson lesions) and in 5 patients – class IV (advanced diabetic glomerulosclerosis).

The expression of ICAM-1, P- and E-selectin was determined using monoclonal antibodies, FITC anti-human CD54 Antibody, FITC anti-human CD62P Antibody, FITC anti-human CD62E Antibody (USA). The intensity of expression in points (0–4), the nature and location of the expression of adhesion molecules in the glomerular endothelium and in peritubular capillaries were assessed.

Results. To identify the prognostic significance of the expression of CD54, CD62R and CD62E in the progression of morphological changes in tissue during the development of DN, correlation analysis and linear regression analysis were performed. The findings demonstrated the role of adhesion molecule expression in the development of mesangial matrix expansion, basement membrane thickening, arteriolar hyplinosis, and tubulointerstitial lesions. The development of morphological changes in tissue is also confirmed by the regression model between the expression of adhesion molecules in tissue with the progression of the stage of DN.

Conclusion. We conducted a statistical analysis to explore how the expression of adhesion molecules affects the changes in kidney tissue. This analysis confirmed the previously suggested idea that interstitial damage plays a crucial role in the progression of DKD. One of the main mechanisms contributing to the development of interstitial fibrosis is the expression of certain adhesion molecules, such as ICAM-1, CD62P, and CD62E

Keywords: Type 2 Diabetes Mellitus, Diabetic Kidney Disease, Renal Biopsy, ICAM-1, P-selectin, E-selectin

Diabetes mellitus is a global chronic disease, the incidence of which is constantly increasing in the world [1, 2]. According to published data from the International Diabetes Federation

(IDF), the incidence of diabetes mellitus is expected to increase by 2040, which could reach 642 million people, and by 2045 this number will increase by 100-250 million people and amount to about 783.2 million [2, 3, 4, 5]. Due to the rapid increase in diabetes mellitus, diabetic nephropathy (DN) is becoming the leading cause of end-stage renal disease (ESRD) worldwide. These patients are twice as high likely to develop CKD as people without diabetes. This is exacerbated by the presence of factors such as hypertension, dyslipidemia, intracranial vascular disease, acute kidney injury (AKI), glomerular atherosclerosis, renal ischemia and nephron loss associated with aging [2]. Consequently, it is not precisely established what the incidence of CKD development is as a result of diabetes. It is believed that 40% of patients with diabetes develop kidney damage. DN is one of the main microvascular complications of diabetes and is characterized by structural and functional changes.

Most of the works published in the world were devoted to the study of glomerular damage in diabetes, which emphasized the role of glomerular damage in the development of pathology [6]. Previously, the prevailing view was that diabetes, especially type 2 diabetes (T2DM), predominantly affects the glomerular structures of the kidney, causing the development of a lesion called DN. However, in 2007, the National Kidney Outcomes Foundation Quality Improvement (NKF/KDOQI) guidelines introduced the term “diabetic kidney disease” (DKD) instead of “diabetic nephropathy” [7]. In 2010, the work of Tervaert T.W.C. et al with a new classification of DN was published, in which the authors described changes in the interstitium, tubules and blood vessels in patients with diabetes against the background of uncharacteristic changes in the glomeruli, such as thickening of glomerular basement membranes, expansion of the mesangial matrix and glomerulosclerosis, which are the main signs of DKD. The authors of the work showed that some patients with diabetes and kidney damage may not have albuminuria, but have impaired renal function and clinical manifestations of tubular dysfunction [8]. Tubulointerstitial lesions in DKD are characterized by inflammation, fibrosis, and loss of renal function, and these lesions may occur earlier than glomerular lesions and play a role in the development and progression of these lesions. Other studies were later published that confirmed these results [9, 10]. Even in DKD patients with primary glomerular disease, renal biopsies have revealed varying degrees of tubulointerstitial damage [11]. Based on the totality of published data worldwide, the American Diabetes Association (ADA) and NKF decided to change the terminology from “diabetic nephropathy” to “diabetic kidney disease” with the intention of more accurately describing the spectrum of kidney dysfunction caused by diabetes [12, 13]. According to Gilbert RE. glomerular changes play a huge role in

the prognosis of kidney damage in patients with diabetes, but are not the main factor that determines the course of the disease. There is evidence that some patients may have no proteinuria and glomerular lesions, but they have reduced renal function, and this may be long before the onset of microalbuminuria and a decrease in glomerular filtration rate [10]. It was shown that in T2DM, 7% of patients had atubular glomeruli, and 26% had abnormalities of the glomerulotubular junction in the absence of significant proteinuria; the degree of such abnormalities inversely correlated with creatinine clearance ($r = -0.70$, $P = 0.011$) [14]. Chevalier R.L. and Forbes M.S. in their work, they demonstrated that damage to the proximal tubules leads to the development of not only podocytopathy, but also to more extensive damage to the glomeruli. In this case, progressive damage to the tubulointerstitium that occurs around atrophic and undifferentiated tubules can directly affect the glomeruli [15].

Glomerular changes include proliferation and hypertrophy of mesangial cells, enlargement of the mesangial matrix, and thickening of the glomerular basement membrane (GBM) with the development of nodular glomerulosclerosis, known as Kimmelstiel-Wilson lesions. The main cause of decreased renal function in patients with DN is changes in the mesangium [16]. Mesangium was first described in 1929 by Zimmerman K. in the form of mesangial cells and mesangial matrix [17]. In 1936, Kimmelstiel P. and Wilson C. in their work first described the formation of nodules that were identified in the renal tissue of patients with diabetes [18]. Each mesangial cell has cytoplasmic processes that extend to the lumen of the capillaries and with their help the cell attaches to the matrix and GBM [19]. Cytoplasmic processes, branching around the walls of the capillaries, form areas of contact between the mesangial processes and endothelial cells. The expansion of the mesangium affects the glomerular capillaries, reduces the filtration surface, narrows or blocks their lumen [20]. The tubulointerstitium develops tubular hypertrophy, thickening of tubular basement membrane (TBM), and interstitial fibrosis, which may be due to epithelial-mesenchymal transformation (EMT), that is, the conversion of tubular cells to interstitial cells. The result of this is the accumulation of extracellular matrix in the interstitium. The main mechanism for the development of tubulointerstitial damage and loss of peritubular capillaries is proteinuria [21, 22].

Long-term diabetes leads to the development of systemic endothelial dysfunction and chronic inflammation, resulting in the development of microvascular complications such as diabetic neuropathy, retinopathy and nephropathy [23]. Numerous studies have been published on the role of adhesion molecules and selectins in the development of DN in patients with diabetes

[24]. An early sign of the development of vascular complications is the adhesion of leukocytes to endothelial cells, through which leukocytes migrate from the lumen of blood vessels to the site of inflammation. The main cell adhesion molecules involved in the development of microvascular complications are vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and selectins [25, 26].

The Aim of the Study.

Research the role of the expression of ICAM-1 (CD54) , P-selectin and E-selectin in the renal tissue of patients with T2DM with different morphological classes of DN and to assess their influence on the development and progression of histological changes in the renal tissue.

Patients and research methods.

The study was conducted in patients with T2DM. Exclusion criteria were: age under 30 years and over 70 years, pregnant women, severe concomitant diseases such as end-stage renal failure, heart failure III-IV class NYHA, history of AMI and stroke, liver dysfunction, malignant neoplasms. As a result, 50 patients suffering from T2DM complicated by the development of DN were selected. The duration of the T2DM was 14.744±0.1.062 years. The average age of the patients was 66.58±3.27 years. There were 35 women, 15 men. The duration of DN from the moment of detection of microalbuminuria to the morphological examination of the renal tissue and diagnosis was 1.65 ± 0.34 years. Diet and metformin were used as hypoglycemic control. All examined patients received complex antihypertensive therapy (including ACE inhibitors or angiotensin II receptor blockers). 63% of patients had coronary heart disease for which they received beta-blockers. All patients underwent a puncture biopsy of the kydney, since an increase in the level of albuminuria was detected in the absence of diabetic retinopathy.

The clinical research carried out in compliance with the WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects, 2013) and the Protocol of Council of Europe Convention on human rights and biomedicine 1999 and articles 20, 22, 23 of the Act “On the basics of healthcare for the Russian Federation citizens” dated November 21, 2011 Fed. Statute №323-FZ (May 26, 2021 edition). The clinical study was conducted in accordance with a procedure approved by the local ethical committee under “Center of Dialysis of St. Petersburg” FRESENIUS MEDICAL CARE. All study participants had signed voluntary informed consent.

Each renal biopsy specimen collected contained at least 10 glomeruli, excluding incomplete glomeruli at the biopsy margin, according to published guidelines [27]. To confirm the morphological diagnosis, light and immunofluorescence microscopy of kidney tissue biopsies were performed. Biopsies were performed by nephrologists under ultrasound guidance. Light microscopy was performed using standard hematoxylin and eosin staining, periodic acid-Schiff stain (PAS), Masson's trichrome, and periodic acid-methenamine silver staining. The assessment of histological changes in renal biopsies was carried out in accordance with the latest international classification of diabetic nephropathy [8].

In 1959, Gellman D.D. et al. first published the results of a correlation analysis of histological changes in renal biopsy samples in 53 patients with DM. However, the proposed assessment of changes in tissue structure has not been used in practical research [28]. Later in 1993, Gambara V. et al. published the results of an analysis of morphological changes in renal tissue in 52 patients with T2DM with severe clinical symptoms [29]. The authors divided patients into three classes based on histological changes and showed no differences in age, average duration of diabetes, renal function, urinary protein excretion and mean arterial pressure among the three classes of patients. In 1996, Fioretto P. et al. published the results of a study of biopsy tissue from 34 patients with non-insulin-dependent DM [30]. The presence of “typical” and “atypical” patterns of renal pathology was shown, which was due to deterioration of metabolism. The authors concluded that hyperglycemia may contribute to the development and progression of tissue morphological changes in elderly patients with non-insulin-dependent DM when compared with a group of younger patients with insulin-dependent DM. Subsequently, it was decided to create a unified classification system for DN in type 1 and type 2 diabetes together, since the histological changes are almost identical, as shown in published studies. That is, in patients with T2DM with progressive DN, the structural and functional relationships are similar to DN in type 1 diabetes and correspond to the general mechanisms of pathogenesis, confirming the important role of tubulointerstitium in the development of renal failure [31, 32]. The creation of a classification of DN was first discussed by the Research Committee of the Renal Society in San Diego in 2006 and again in September 2008 in Leiden. In 2010, the classification was accepted and published [8]. According to the accepted classification, four classes of DN are identified.

Class I: Glomerular Basement Membrane Thickening. GBM thickening, which is a characteristic feature and correlates with disease duration [33]. In the absence of mesangial expansion, nodules in the mesangial matrix (Kimmelstiel-Wilson lesions), and global

glomerulosclerosis of more than 50% of the glomeruli, the biopsy is classified as Class I. GBM thickness averages 430 nm in men and 395 nm in women. These extreme levels are described as “prediabetic” lesions: patients with proteinuria and isolated GBM thickening, but without overt diabetes. This group includes cases that have been called “normal or near-normal DN” [34].

Class II: Mesangial expansion, mild (IIa) or severe (IIb). Mesangial expansion is an increase in extracellular material in the mesangium to an increase in the width of the intercellular space, exceeding two nuclei of mesangial cells in the two lobes of the glomerulus. The difference between mild and severe mesangial expansion is based on whether the mesangial expansion is smaller or larger than the average capillary lumen area. Moderate mesangial expansion in more than 25% of the total mesangium corresponds to class IIa. Severe mesangial expansion in more than 25% of the total mesangium, biopsy is classified as IIb. Class II includes any expansion of the mesangium—mesangial hypercellularity, matrix expansion, or “mesangiosclerosis.”

Class III: Nodular Sclerosis (Kimmelstiel–Wilson lesions). The presence of one Kimmelstiel–Wilson lesion in the biopsy specimen and no more than 50% total glomerulosclerosis is classified as class III. Kimmelstiel–Wilson lesions appear in T1DM and T2DM as focal, lobular, round-oval mesangial lesions with an acellular hyaline/matrix core surrounded peripherally by sparse crescent-shaped mesangial nuclei [35]. In the initial stage of development of nodular sclerotic lesions, two important processes occur: mesangiolysis and detachment of endothelial cells from the GBM, which leads to disruption of the connection between the mesangial region and the GBM and precedes the development of Kimmelstiel-Wilson lesions, which consist of an accumulation of mesangial matrix with collagen fibrils, small lipid particles and cellular debris. The formed Kimmelstiel–Wilson lesion destroys the normal structure of the glomerulus and reduces the number of mesangial cells. The nodules are distributed in a horseshoe-shaped area corresponding to the peripheral or intralobular mesangium. The occurrence of Kimmelstiel–Wilson lesions is considered to be transitional from an early or moderate stage to disease progression [36].

Class IV: Advanced Diabetic Glomerulosclerosis. Class IV implies a progressive course of DN with more than 50% of cases of total glomerulosclerosis, with clinical or pathological evidence that sclerosis is associated with DN. Glomerulosclerosis in DN is the endpoint of multifactorial mechanisms that lead to excessive accumulation of extracellular matrix proteins, such as collagen types I, III and IV, and fibronectin in the mesangial space, ultimately leading

to glomerulosclerosis [37]. Nodular changes in the glomeruli were first described in 1936 in 8 patients with diabetes [18]. In 1941, Allen A., after analyzing 105 biopsies of renal tissue, proved the connection between the previously described nodal changes and diabetes [38].

Light microscopy of kidney biopsy tissue was assessed using the following indicators: the presence of global and segmental glomerular sclerosis, glomerular cellularity, the severity of mesangial matrix expansion (less than and more than 25%), GBM thickening, Kimmelstiel-Wilson nodular formations, periglomerular sclerosis, sclerotic changes in the interstitium, the presence and the severity of mononuclear inflammatory infiltrates in the interstitium, the presence of protein masses in the lumens of the tubules, atrophy and dystrophy of the epithelium of the urinary tubules, hyalinosis of afferent and efferent arterioles. The severity of morphological changes was assessed using a semi-quantitative method in points (0–3). Global and segmental glomerular sclerosis was assessed as the percentage of globally and segmentally sclerotic glomeruli from the total number of glomeruli in the nephrobiopsy section. Interstitial fibrosis and tubular atrophy (IFTA) was assessed as a percentage of the total interstitial and tubular area affected. Score 0 - absence of IFTA in the biopsy, score 1 - IFTA less than 25%, score 2 - 25%, but less than 50% of biopsies have IFTA, score 3 - IFTA at least 50% [39]. Mononuclear infiltration (IM), afferent and efferent hyalinosis (AH) were also scored (0–2 and 0–2, respectively) according to the criteria of the international classification of DN23 [8].

According to light microscopy, class IIa (mild mesangial expansion) was detected in 12 patients (24%), class IIb (severe mesangial expansion) was detected in 14 patients (28%), in 19 patients (38%) – class III (nodular Kimmelstiel-Wilson lesions), in 5 patients (10%) – class IV (advanced diabetic glomerulosclerosis). Based on the results of light microscopy, no evidence of glomerulonephritis was obtained in any patient.

In all patients, the expression of selectins CD62P (P-selectin) and CD62E (E-selectin) in the kidney tissue was determined using monoclonal antibodies labeled FITC anti-human CD62P (P-Selectin) Antibody, clone AK4 Cat#304904 and FITC anti-human CD62E (E-Selectin) Antibody, clone HCD62E Cat# 322606 and intercellular adhesion molecule-1 (ICAM-1), using monoclonal antibodies FITC anti-human CD54 Antibody, clone HCD54 Cat# 322720 (Biolegend, USA).

Statistical analysis.

Statistical processing of the obtained data was carried out using the IBM SPSS Statistics software package, version 26 (Armonk, NY: IBM Corp.). Group results are presented as mean ± standard error (M ± Standard Error), standard deviation (SD), and median (interquartile range) for continuous variables. Statistical comparison of data between groups of patients was carried out using the nonparametric Mann–Whitney U test. Differences in continuous variables between the two groups were assessed using the independent sample Student's t test and were considered significant if $p \leq 0.05$. For statistical processing, parametric (Pearson's method) and non-parametric (Spearman's test, Kendall's tau (τ) test) methods were used. To verify compliance with the condition of independence of observations, linear regression analysis was carried out (with the calculation of the coefficient of determination (R Square) and the Durban–Watson test) and analysis of variance (ANOVA Analisis of Variance) with the calculation of the Fisher test (F) to test the significance of the model. The standardized ß coefficient with 95% confidence intervals was calculated. The critical level of significance of the difference in indicators was taken equal to 0.05

Results. Table 1 presents the clinical characteristics of patients with T2DM who underwent renal tissue biopsy.

Table 1. Clinical characteristics of patients with T2DM with DN

Characteristic	**Patients**
Number of patients	50
Female /Male	35/15
Age (years)	66,582±3,271 (95% CI: 62,355 – 68,758)
Duration of T2DM (years)	14,744±1,062 (95% CI: 12,809 - 17,020)
Duration of DN (years)	1,650±0,341 (95% CI: 1,386 - 1,861)
Creatinine (μmol/l)	106,400 ±7,521 (95% CI: 93,260 - 122,612)
GFR CKD-EPI (ml/min)	69,445 ±4,684 (95% CI: 60,097 - 78,540)
Albuminuria (mg/day)	1143,020 ± 316,012 (95% CI: 597,304 – 1789,044)
SBP (mmHg)	142,936 ±2,312 (95% CI: 138,511 - 147,468)
DBP (mmHg)	80,021±1,018 (95% CI: 78,043 - 82,063)
HbA1c (%)	7,884±0,420 (95% CI: 7,080 - 8,695)

Retinopathy	No

The expression of selectins CD62P and CD62E, intercellular adhesion molecule-1 (ICAM-1) in the kidney tissue was analyzed in the general group of patients (Figure 1).

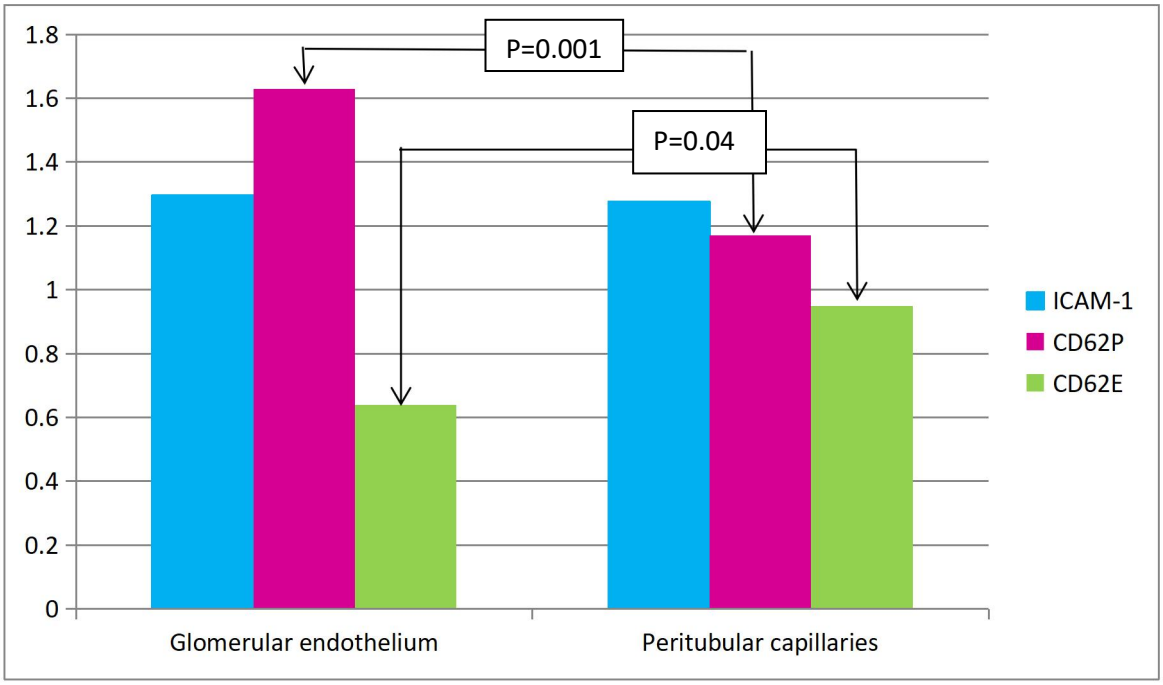

Figure 1. Intensity of expression of ICAM-1, CD62P, CD62E in renal tissue in T2DM patients with DN.

Figure 1 shows, that the expression of ICAM-1, CD62P, CD62E was detected both in the glomerular endothelium and peritubular capillaries. The expression of CD62P was significantly higher in the glomerular endothelium, and the expression of CD62E in the peritubular capillaries. ICAM-1 expression was almost identical in both zones. Next, we analyzed the expression of ICAM-1, CD62P and CD62E separately in each class of DN (Table 2).

Table 2. Intensity of expression of ICAM-1, CD62P, CD62E in renal tissue in different groups of patients with T2DM with DN

IIa class (n = 12) (1)	IIb class (n = 14) (2)	III class (n = 19) (3)	IV class (n = 5) (4)	P
ICAM-1 expression in glomerular endothelium				

1,500 ±0,372 (95% CI: 0,800 – 2,200)	1,142 ± 0,261 (95% CI: 0,642 – 1,642)	1,000 ± 0,208 (95% CI: 0,613 – 1,421)	0,500± 0,227 (95% CI: 0,083 – 1,000)	P1,2=0,05 P1,3=0,01 P1,4=0,001 P2,3=0,08 P2,4=0,04 P3,4=0,05
ICAM-1 expression in peritubular capillaries				
1,400±0,380 (95% CI: 0,700 - 2,100)	1,285 ±0,238 (95% CI:0,857 – 1,785)	1,157 ±0,238 (95% CI:0,857 – 1,785)	0,750±0,207 (95% CI: 0,333 – 1,166)	P1,2=0,07 P1,3=0,06 P1,4=0,001 P2,3=0,07 P2,4=0,05 P3,4=0,06
CD62P expression in glomerular endothelium				
3,500±2,088 (95% CI:-1,224 - 8,224)	1,500±0,291 (95% CI: 0,869 – 2,130)	1,000±0,253 (95% CI:0,467 – 1,532)	0,000	P1,2=0,04 P1,3=0,001 P1,4 =0,0001 P2,3 =0,04 P2.4 =0,0001 P3,4=0,0001
CD62P expression in peritubular capillaries				
1,600±0,266 (95% CI: 0,996 - 2,203)	1,214±0,280 (95% CI:0,607 – 1,821)	0,842±0,191 (95% CI:0,440 – 1,244)	0,297±0,109 (95% CI: 0,106 – 0,531)	P1,2 =0,05 P1,3 =0,001 P1,4=0,001 P2,3=0,05

				P2,4=0,001 P3,4=0,05
CD62E expression in glomerular endothelium				
0,900±0,349 (95%CI: 0,200–1,600)	0,428±0,226 (95% CI:0,063 - 0,920)	0,421±0.183 (95% CI: 0,157 – 0,736)	0,000	P1,2=0,001 P1,3 =0,001 P1,4=0,0001 P2,3=0,07 P2,4=0,0001 P3,4=0,0001
CD62E expression in peritubular capillaries				
1,400±0,452 (95% CI: 0,600 - 2,203)	0,785±0,309 (95% CI: 0,2143 – 1,498)	0,684±0,212 (95% CI: 0,315 – 1,157)	0,195±0.088 (95% CI: 0,042 – 0,383)	P1,2=0,001 P1,3=0,001 P1,4=0,0001 P2,3=0,08 P2,4=0,004 P3,4=0,002

From the data presented in the table it can be seen that the intensity of expression of ICAM-1, CD62P, CD62E decreases with the progression of histological changes in the tissue. Next, a correlation analysis was carried out between the expression of ICAM-1, CD62P, CD62E in tissue and the duration of T2DM in patients. No reliable correlations were identified. But correlations with the DN class were identified (Table 3).

Table 3 Correlations between the expression of CD62p and ICAM-1 in the glomerular endothelium with DN class in the general group of T2DM patients

Correlations		
Kendall(τ)	Spearman (r)	Pearson (R)
CD62P ***expression in glomerular endothelium***		

τ= -0,345 p=0,007	r= - 0,380 p=0,008	R= - 0,383 =0,008
ICAM-1 expression in glomerular endothelium		
τ=-0,261 p=0,038	r= - 0,300 p=0,041	R = - 0,346 p= 0,017

Correlation analysis between the expression of ICAM-1, CD62P, CD62E in renal tissue with morphological changes in the general group of patients showed the following results (Table 4).

Table 4 Correlations between the expression of ICAM-1, CD62P, CD62E in kidney tissue with morphological changes in the general group of patients with DN

Morphological changes	**Correlations**		
	Kendall(τ)	**Spearman (r)**	**Pearson (R)**
ICAM-1 expression in glomerular endothelium			
Mesangial matrix expansion	τ= -0,339 p=0,011	r= -0,373 p=0,010	R = -0,398 p=0,006
ICAM-1 ***expression in peritubular capillaries***			
Periglomerular sclerosis	τ=0,281 p=0,038	r=0,310 p=0,036	-
CD62P ***expression in glomerular endothelium***			
Basement membrane thickening	τ=-0,289 p=0,029	r=-0,319 p=0,029	R=-0,357 =0,014
Kimmelstiel–Wilson lesions	τ=-0,289 p=0,26	r=-0,325 p=0,025	R =-0,326 p= 0,025
Arteriole hyalinosis	τ= -0,316 p=0,015	r = -0,378 p=0,009	R =-0,340 p=0,019

CD62E ***expression in glomerular endothelium***			
Kimmelstiel–Wilson lesions	τ= - 0,289 p=0,026	r= -0,325 p=0,026	R = - 0,300 p=0,040
Arteriole hyalinosis	τ= - 0,298 p=0,026	r= - 0,355 p=0,014	R = - 0,324 p=0,026

The table data shows that the expression of all studied molecules in renal tissue correlates with the development of morphological changes in renal tissue. A Pearson correlation was also found between the duration of T2DM and:

• thickening of the basement membrane R=0.355 p=0.016;

• Kimmelstiel-Wilson lesions R=0.314 p=0.033

• mesangial expansion R=0.304 p=0.040

In order to identify the prognostic significance of the expression of ICAM-1, CD62P, CD62E and their role in the progression of morphological changes in renal tissue during the development of DN, linear regression analysis was carried out with the calculation of determination coefficients R2 (R Square) and analysis of variance (ANOVA Analysis of Variance) using F test with 95% confidence interval. The obtained values, indicating the significance of the regression models, are presented below (Table 5, 6).

Table 5 Regression models of the significance of ICAM-1 expression in kidney tissue in the general group of patients with DN

Morphological changes	Coefficient of determination (R2)	Standardized coefficient (ß)	Fisher test (F)	P
ICAM-1 expression in glomerular endothelium				
Mesangial matrix expansion	0,532	β=0,732	53,059	0,000
Basement membrane	0,520	β=0,720	49,919	0,000

thickening				
Arteriole hyalinosis	0,445	β=0,670	38.644	0,000
ICAM-1 expression in peritubular capillaries				
Interstitial sclerosis	0,457	β=0,685	40,646 64,825	0,000
Atrophy of tubular epithelium	0,548	β=0,740	55,603	0,000

From the presented results it is clear that increased expression of ICAM-1 in the glomerular endothelium is associated with the severity of expansion of the mesangial matrix, thickening of basement membrane and arteriolar hyalinosis. Increased expression of ICAM-1 in peritubular capillaries is associated with the development of interstitial sclerosis and tubular epithelial atrophy.

Table 6 Regression models of the significance of CD62p, CD62e expression in kidney tissue in the general group of patients with DN

Morphological changes	Coefficient of determination (R^2)	Fisher test (F)	p
CD62P expression in glomerular endothelium			
Mesangial matrix expansion	0,317	21,317	0,000
Arteriole hyalinosis	0,213	12,476	0.001
Basement membrane thickening	0,255	15,708	0.000
CD62E expression in glomerular endothelium			
Mesangial matrix expansion	0,206	11,926	0,001

Basement membrane thickening	0,216	13,926	0,001
Arteriole hyalinosis	0,206	13,202	0.001
CD62P expression in peritubular capillaries			
Atrophy of tubular epithelium	0,558	6,632	0.013
Interstitial sclerosis	0,549	54,732	0,0001
CD62E expression in peritubular capillaries			
Atrophy of tubular epithelium	0,356	25,462	0,0001
Interstitial sclerosis	0,338	22,932	0,0001

From the presented data it follows that the expression of selectins CD62P and CD62E influences the development and progression of morphological changes in renal tissue in patients with DN.

The development of morphological changes in tissue is also confirmed by the regression model between the expression of adhesive molecules in tissue with the progression of the stage of DN (Table 7).

Table 7. Regression models of the significance of the expression of ICAM-1, CD62P and CD62E in the glomerular capillary endothelium on progression of the DN stage in the general group of patients

Glomerular endothelium	Coefficient of determination (R^2)	Fisher test (F)	p
ICAM-1 expression	0,430	36,420	0,000
CD62P expression	0,216	12,684	0,001
CD62E expression	0,204	11,779	0,001

Discussion.

The main cell adhesion molecules involved in the development of microvascular complications are VCAM-1, ICAM-1 and selectins (E-selectin, L-selectin and P-selectin). These molecules contribute to the development of endothelial dysfunction, micro- and macrovascular complications [40]. Further, leukocyte adhesion to the endothelium is mediated by ICAM-1 and VCAM-1 [41]. The first work investigating the role of ICAM-1 expression in biopsy tissue from five normal kidneys and 47 kidney biopsies with different morphological diseases (rapidly progressive glomerulonephritis, mesangioproliferative glomerulonephritis, IgA nephropathy, Henoch-Schönlein purpura, lupus nephritis and focal segmental glomerulosclerosis) was published group of authors in 1991, who showed that 59% of mononuclear infiltrate cells expressed ICAM-1 in all forms of glomerulonephritis [42]. Further study of the expression of adhesion molecules in renal tissue of pre-transplant biopsies (n = 20) and renal transplant biopsies (n = 42) showed a correlation between the number of infiltrating leukocytes and the magnitude of the expression of adhesion molecules ICAM-1, VCAM-1 and E-selectin on endothelial cells. Differences in endothelial expression of ICAM-1 and VCAM-1 in the proximal tubule were identified. The authors of the work suggested that the induction of the expression of adhesion molecules is associated with focal leukocyte infiltration [43]. In 1994, data were published on the relationship between interstitial ICAM-1 expression and interstitial leukocyte infiltration and tubulointerstitial damage [44]. The authors concluded that glomerular expression of adhesion molecules does not play an important role in the development of tubulointerstitial damage. And other data were obtained in 1997 by Sugimoto N. et al.: in the glomeruli of the kidney taken from rats with diabetes induced by streptozotocin, increased expression of ICAM-1 was shown at the early stage of the disease. The authors concluded that activation of ICAM-1 promotes the recruitment of monocytes/macrophages into diabetic glomeruli, and glomerular hypercellularity induces ICAM-1 expression in diabetic nephropathy [45]. Mononuclear cells infiltrating the glomeruli begin to produce various cytokines and growth factors that contribute to damage to the glomerulus and changes in its structure. When analyzing our data, we were unable to obtain confirmation of the connection between the expression of ICAM-1 in the glomerular endothelium and peritubular capillaries with the presence of mononuclear infiltration ($R2 = 0.053$ $\beta = 0.231$ $F = 2.539$ $p = 0.118$; and $R2 = 0.028$ $\beta = -0.169$ $F = 1.316$ $p = 0.257$, respectively), which coincides with the data published by Roy-Haudhury Prabir and colleagues, which did not reveal a significant correlation between glomerular expression of

ICAM-1 and glomerular macrophage infiltration [46]. We did not reveal a correlation between the expression of P- and E-selectins in the glomerular endothelium with the development of tubulointerstitial sclerosis.

Another most comprehensive studies of the expression of ICAM-1, P- and E-selectins in kidney tissue was published by Roy-Chaudhury Prabir et al. in 1996 [46]. The study was conducted on 119 biopsy blocks of kidneys taken from patients with different morphological diagnoses. The expression of P- and E-selectins on extraglomerular vascular endothelium was assessed, and a correlation of selectin expression with glomerular cellular infiltration was shown. The expression of P-selectins and E-selectins was significantly increased ($P < 0.0001$ and $P < 0.0001$, respectively) in the glomerular endothelium. It has also been shown that the expression of ICAM-1, P- and E-selectin molecules in the tubulointerstitium is associated with interstitial fibrosis and tubular atrophy and may contribute to the progression of kidney disease. There was a positive Spearman correlation between tubular atrophy and interstitial fibrosis and the expression of ICAM-1 ($r = 0.61$, $P < 0.0001$), E-selectin ($r = 0.71$, $P < 0.0001$) and P-selectin ($r = 0.72$, $P < 0.0001$). Spearman correlations were found regardless of the morphological form of the primary diagnosis, but were clearly associated with histological damage. The authors of the work showed that there is a common pathway of tubulointerstitial damage, regardless of the primary diagnosis, and the expression of adhesion molecules within the tubulointerstitium may be an important mechanism in the pathogenesis of DN [46]. Our work revealed a correlation between the expression of ICAM-1 in peritubular capillaries and the development of tubulointerstitial sclerosis in patients with type 2 diabetes ($\tau=0.281$ $p=0.038$; and $r=0.310$ $p=0.036$; respectively). The linear regression method demonstrated an associative relationship between the expression of ICAM-1 in peritubular capillaries and the development of tubular epithelial atrophy ($R2=0.548$) and tubulointerstitial sclerosis ($R2=0.457$); expression of CD62P in peritubular capillaries is associated with the development of tubular epithelial atrophy ($R2 = 0.558$) and tubulointerstitial sclerosis ($R2 = 0.549$); expression of CD62E in peritubular capillaries - with tubular epithelial atrophy ($R2 =0.356$) and tubulointerstitial sclerosis ($R2 =0.338$). Also, the mechanism of tubulointerstitial damage and progression of DN is confirmed by our regression model of the relationship between the expression of ICAM-1, CD62R and CD62E with the progression of the stage of DN in the general group of patients. Taken together, these data support the hypothesis of Roy-Chaudhury Prabir et al. about a unified mechanism of tubulointerstitial damage and the

significant role of the expression of ICAM-1, CD62P and CD62E molecules in its development.

Omoto S., et al. published data on increased levels of CD62P expression in the glomeruli and interstitium of renal tissue in patients with diabetes [47]. Hirata K., et al. showed significantly increased levels of selectin expression in both the glomeruli and the interstitium of renal tissue from patients with DKD compared with a group of patients with other diseases (including minimal change nephrotic syndrome, membranous nephropathy, IgA nephropathy, mesangioproliferative glomerulonephritis and lupus nephritis) [48]. The method of regression analysis of the expression of adhesion molecules in the glomeruli revealed the influence of the expression of ICAM-1, CD62P and CD62E on the development of basement membrane thickening, mesangial expansion and arteriolar hyalinosis in patients with DN. Thus, in our work, we showed statistically significant histological changes in the glomeruli under the influence of the expression of ICAM-1, CD62P and CD62E in patients with DN. The earliest histological change is thickening of the GBM. 5-7 years after the onset of diabetes, patients develop mesangial expansion [49]. We confirmed this by obtaining a Pearson correlation between GBM thickening, Kimmelstiel-Wilson nodules , and mesangial expansion with the duration of type 2 diabetes.

Conclusion.

We conducted a statistical analysis to explore how the expression of adhesion molecules affects the changes in kidney tissue. This analysis confirmed the previously suggested idea that interstitial damage plays a crucial role in the progression of DKD. One of the main mechanisms contributing to the development of interstitial fibrosis is the expression of certain adhesion molecules, such as ICAM-1, CD62P, and CD62E.

Funding. The study had no sponsorship

Conflict of interest. The authors declare no conflict of interest

Authors' contribution.

conception and research design –., Ryabova T. S., Rakityanskaya I. A.;

material gathering and processing – Ryabova T. S.. Rakityanskaya I. A.;

data analysis and interpretation – Rakityanskaya I. A., Ryabova T. S.;

lab research – Rakityanskaya I. A.;

statistical processing of data –Rakityanskaya I. A., Ryabova T. S.;

script composition – Ryabova T. S., Rakityanskaya I. A.;

editing – Ryabova T. S.;

research supervision – Rakityanskaya I.A.;

text writing and editing – Rakityanskaya I. A., Ryabova T. S.;

responsibility for integrity of all article's parts – Rakityanskaya I.A.;

Script further revision for important intellectual content – Rakityanskaya I. A., Ryabova T. S.

All the authors have made substantial contribution to this study and approved final script version.

References

[1] GBD 2021 Diabetes Collaborators . Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: A systematic analysis for the Global Burden of Disease Study 2021. Lancet (2023) 402:203–34. doi: 10.1016/S0140-6736(23)01301-6

[2] Selby N.M., Selby N.M., Taal M.W. An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines // Diabetes Obes. Metab. 2020; 22(1): 3-15. doi: 10.1111/dom.14007.

[3] Alicic R.Z., Rooney M.T., Tuttle K.R. Diabetic Kidney Disease: Challenges, Progress, and Possibilities. Clin J Am Soc Nephrol. 2017 Dec 7;12(12):2032-2045. doi: 10.2215/CJN.11491116.

[4] Gheith Osama , Farouk Nashwa , Nampoory Narayanan , Medhat A Halim, Torki Al-Otaibi. Diabetic kidney disease: world wide difference of prevalence and risk factors J Nephropharmacol. 2015 Oct 9;5(1):49-56. eCollection 2016.

[5] Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence

estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022 Jan;183:109119. doi: 10.1016/j.diabres.2021.109119

[6] Chengren Xu, Xiaowen Ha, Shufen Yang, Xuefei Tian, Hong Jian. Advances in understanding and treating diabetic kidney disease: focus on tubulointerstitial inflammation mechanisms Front Endocrinol (Lausanne). 2023; 14: 1232790. doi: 10.3389/fendo.2023.1232790

[7] Levin A., Rocco M., Eknoyan G., Levin N., Becker B., Blake P.G, et al. . KDOQI clinical practice guidelines and clinical practice recommendations for diabetes and chronic kidney disease. Am J Kidney Dis (2007) 49:S12–S154. doi: 10.1053/j.ajkd.2006.12.005

[8] Tervaert T.W.C., Mooyaart A.L., Amann K., Cohen A.H., Cook H.T., Drachenberg CB, et al.. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol (2010) 21:556–63. doi: 10.1681/ASN.2010010010

[9] Zeni L., Norden A.G.W., Cancarini G., Unwin R.J. A more tubulocentric view of diabetic kidney disease. J Nephrol (2017) 30:701–17. doi: 10.1007/s40620-017-0423-9,

[10] Gilbert Richard E. Proximal Tubulopathy: Prime Mover and Key Therapeutic Target in Diabetic Kidney Disease. Diabetes . 2017 Apr;66(4):791-800. doi: 10.2337/db16-0796

[11] Klessens C.Q.F., Woutman T.D., Veraar K.A.M., Zandbergen M., Valk E.J.J., Rotmans J.I., et al.. An autopsy study suggests that diabetic nephropathy is underdiagnosed. Kidney Int (2016) 90:149–56. doi: 10.1016/j.kint.2016.01.023

[12] Martínez-Castelao A., Navarro-González J., Górriz J., De Alvaro F. The concept and the epidemiology of diabetic nephropathy have changed in recent years. JCM (2015) 4:1207–16. doi: 10.3390/jcm4061207,

[13] Tuttle K.R., Bakris G.L., Bilous R.W., Chiang J.L., De Boer I.H., Goldstein-Fuchs J, et al.. Diabetic kidney disease: A report from an ADA consensus conference. Am J Kidney Dis (2014) 64:510–33. doi: 10.1053/j.ajkd.2014.08.001

[14] White K.E., Marshall S.M., Bilous R.W. Prevalence of atubular glomeruli in type 2 diabetic patients with nephropathy. Nephrol Dial Transplant 2008 Nov;23(11):3539-45. doi: 10.1093/ndt/gfn351.

[15] Chevalier R.L., Forbes M.S. Generation and evolution of atubular glomeruli in the progression of renal disorders. J Am Soc Nephrol. 2008 Feb;19(2):197-206. doi: 10.1681/ASN.2007080862..

[16] Steffes M.W., Osterby R., Chavers B., Mauer S.M: Mesangial expansion as a central mechanism for loss of kidney function in diabetic patients. Diabetes 1989 Sep;38(9):1077-81. doi: 10.2337/diab.38.9.1077.

[17] Zimmerman K. Über den bau des glomerulus der säugerniere. Weitere mittleillungen. Z Mikrosk Anat Forsch. 1933;32:176–278

[18] Kimmelstiel P., Wilson C. Intercapillary lesions in the glomeruli of the kidney. Am J Pathol. 1936;12(1):83–98

[19] Davies M. The mesangial cell: A tissue culture view. Kidney Int. 1994;45(2):320–7. doi: 10.1038/ki.1994.41

[20] Sakai F., Kriz W. The structural relationship between mesangial cells and basement membrane of the renal glomerulus. Anat Embryol. 1987;176(3):373–86. doi: 10.1007/BF00310191

[21] Nangaku M. Mechanisms of tubulointerstitial injury in the kidney: final common pathways to end-stage renal failure. Intern. Med. 2004; Jan;43(1):9-17. doi: 10.2169/internalmedicine.43.9.

[22] Zavadil J., Bottinger E.P. TGF-βand epithelial-to-mesenchymal transitions. Oncogene 2005 Aug 29;24(37):5764-74. doi: 10.1038/sj.onc.1208927.

[23] American Diabetes Association . Diagnosis and classification of diabetes mellitus. Diabetes Care 2014; 37(1):S81–90. doi: 10.2337/dc14-S081

[24] Raimund Pichler, Maryam Afkarian, Brad P. Dieter, Katherine R. Tuttle. Immunity and inflammation in diabetic kidney disease: translating mechanisms to biomarkers and treatment targets. Am J Physiol Renal Physiol. 2017; Apr 1;312(4):F716-F731. doi: 10.1152/ajprenal.00314.2016

[25] Blum A., Pastukh N., Socea D., Jabaly H. Levels of adhesion molecules in peripheral blood correlat with stages of diabetic retinopathy and may serve as bio markers for microvascular complications. Cytokine 2018; 106:76–9. doi: 10.1016/j.cyto.2017.10.014,

[26] Liu J.J., Yeoh L.Y., Sum C.F., Tavintharan S., Ng XW, Liu S, et al.. Vascular cell adhesion molecule-1, but not intercellular adhesion molecule-1, is associated with diabetic kidney disease in asians with type 2 diabetes. J Diabetes Complications 2015; 29:707–12. doi: 10.1016/j.jdiacomp.2015.02.011

[27] Solez K., Colvin R.B., Racusen L.C,. Haas M., Sis B., Mengel M., et al. Banff 07 classification of renal allograft pathology: Updates and future directions. Am J Transplant 2008; 8(4):753-60. doi: 10.1111/j.1600-6143

[28] Gellman D.D., Pirani C.L., Soothill J.F., Muehrcke R.C., Kark R.M. Diabetic nephropathy: A clinical and pathologic study based on renal biopsies. Medicine (Baltimore) 1959 Dec:38:321-67.

[29] Gambara V., Mecca G., Remuzzi G., Bertani T: Heterogeneous nature of renal lesions in type II diabetes. J Am Soc Nephrol 1993; Feb;3(8):1458-66. doi: 10.1681/ASN.V381458.

[30] Fioretto P., Mauer M., Brocco E., Velussi M., Frigato F., Muollo B., et al: Patterns of renal injury in NIDDM patients with microalbuminuria. Diabetologia 1996; Dec;39(12):1569-76. doi: 10.1007/s001250050616.

[31] Osterby R., Gall M.A., Schmitz A., Nielsen F.S., Nyberg G., Parving H.H: Glomerular structure and function in proteinuric type 2 (non-insulin-dependent) diabetic patients. Diabetologia 1993; Oct;36(10):1064-70. doi: 10.1007/BF02374500.

[32] White K.E., Bilous R.W.: Type 2 diabetic patients with nephropathy show structural-functional relationships that are similar to type 1 disease. J Am Soc Nephrol 2000; Sep;11(9):1667-1673. doi: 10.1681/ASN.V1191667.

[33] Perrin N.E., Torbjornsdotter T.B., Jaremko G.A., Berg U.B.: The course of diabetic glomerulopathy in patients with type I diabetes: A 6-year follow-up with serial biopsies. Kidney Int 2006; Feb;69(4):699-705. doi: 10.1038/sj.ki.5000146.

[34] Haas M. Alport syndrome and thin glomerular basement membrane nephropathy: A practical approach to diagnosis. Arch Pathol Lab Med 2009; Feb;133(2):224-32. doi: 10.5858/133.2.224.

[35] Stout L.C., Kumar S., Whorton E.B. Focal mesangiolysis and the pathogenesis of the Kimmelstiel–Wilson nodule. Hum Pathol 1993; Jan;24(1):77-89. doi: 10.1016/0046-8177(93)90066-p.

[36] Hong D., Zheng T., Jia-qing S., Jian W., Zhi-hong L., Lei-shi L: Nodular glomerular lesion: A later stage of diabetic nephropathy? Diabetes Res Clin Pract. 2007; Nov;78(2):189-95. doi: 10.1016/j.diabres.2007.03.024.

[37] Qian Y., Feldman E., Pennathur S., et al. From fibrosis to sclerosis: Mechanisms of glomerulosclerosis in diabetic nephropathy. Diabetes 2008; Jun;57(6):1439-45. doi: 10.2337/db08-0061.

[38] Allen A: So-called intercapillary glomerulosclerosis: A lesion associated with diabetes mellitus. Morphogenesis and significance. Arch Path 32: 33–51, 1941

[39] Najafian B., Kim Y., Crosson J.T., et al. Atubular glomeruli and glomerulotubular junction abnormalities in diabetic nephropathy. J Am Soc Nephrol 2003; 14: 908–917, doi: 10.1097/01.asn.0000057854.32413.81.

[40] Blum A., Pastukh N., Socea D., Jabaly H. Levels of adhesion molecules in peripheral blood correlat with stages of diabetic retinopathy and may serve as bio markers for microvascular complications. Cytokine 2018; 106:76–9. doi: 10.1016/j.cyto.2017.10.014

[41] Siddiqui Khalid, George Teena P., Mujammami Muhammad et al. The association of cell adhesion molecules and selectins (VCAM-1, ICAM-1, E-selectin, L-selectin, and P-selectin) with microvascular complications in patients with type 2 diabetes: A follow-up study. Front Endocrinol (Lausanne) 2023; Feb 9:14:1072288. doi: 10.3389/fendo.2023.1072288

[42] Lhotta K., Neumayer H.P, Joannidis M., Geissler, Königet P.. Renal expression of intercellular adhesion molecule-1 in different forms of glomerulonephritis. Clin Sci (Lond). 1991; Oct;81(4):477-81. doi: 10.1042/cs0810477.

[43] Fuggle S.V., Sanderson J.B., Gray D., Richardson W.A., Morriset P.J. . Variation in expression of endothelial adhesion molecules in pretransplant and transplanted kidneys—Correlation with intragraft events. Transplantation 1993; Jan;55(1):117-23. doi: 10.1097/00007890-199301000-00022.

[44] Nikolic-Paterson D.J., Lan H.Y., Hill P.A., Vannice J.L., Atkinset R.C. Suppression of experimental glomerulonephritis by the interleukin-1receptor antagonist: Inhibition of intercellular adhesion molecule-1 expression. JAm Soc Nephrol 1994; Mar;4(9):1695-700. doi: 10.1681/ASN.V491695.

[45] Sugimoto H., Shikata K., Hirata K., Akiyama K., Matsuda M., Kushiro M., et al. Increased expression of intercellular adhesion molecule-1 (ICAM-1) in diabetic rat glomeruli: glomerular hyperfiltration is a potential mechanism of ICAM-1 upregulation. Diabetes 1997; Dec;46(12):2075-81. doi: 10.2337/diab.46.12.2075.

[46] Roy-haudhury Prabir, Wu B.R., King G., Campbell M., Macleod A.M. et al.,. Adhesion molecule interactions in human glomerulonephritis: Importance of the tubulointerstitium. Kidney International 1996; Jan;49(1):127-34. doi: 10.1038/ki.1996.17.

[47] Omoto S., Nomura S., Shouzu A., Hayakawa T., Shimizu H., Miyake Y., et al.,. Significance of Platelet-Derived Microparticles and Activated Platelets in Diabetic Nephropathy. Nephron Exp. Nephrol. 1999;81:271–277. doi: 10.1159/000045292

[48] Hirata K., Shikata K., Matsuda M., Akiyama K., Sugimoto H., Kushiro M., et al.,. Increased expression of selectins in kidneys of patients with diabetic nephropathy. Diabetologia. 1998;41:185–192. doi: 10.1007/s001250050888

[49] Alicic R.Z., Rooney M.T., Tuttle K.R. Diabetic kidney disease: Challenges, progress, and possibilities. Clin J Am Soc Nephrol. 2017;12(12):2032–45. doi: 10.2215/CJN.11491116.