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The role of inflammatory markers and growth factors in progression of chronic kidney disease in patients with diabetes mellitus

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ABSTRACT

Introduction: A Diabetes mellitus (DM) and chronic kidney disease (CKD) are two chronic non-communicable diseases that have exceeded epidemic thresholds in all countries of the world. The problem of early diagnosis, prevention and treatment of CKD continues to be relevant for modern medicine, and assessment of the severity of CKD and associated cardiovascular complications is of great practical importance for primary and secondary prevention.

Objectives: The aim of the study was to assess the role of proinflammatory cytokines, chemokines and growth factors in progression of CKD in patients with DM.

Patients and Methods: We screened 155 type 2 DM patients aged 65.00 (55.00-71.00) years. Control group included 21 healthy people with the same age. Renal function was assessed based on the levels of serum creatinine, cystatin C, estimated glomerular filtration rate (eGFR), which was calculated according to the CKD-EPI (chronic kidney disease epidemiology collaboration) equation, and albuminuria, which was assessed as albumin/creatinine ratio (A/C). The analysis of serum and urine biomarkers was carried out by enzyme immunoassay using commercial test systems.

Results: Patients with DM had significantly higher levels of proinflammatory cytokines in comparison with the control group. The levels of interleukin-6 (IL-6), high-sensitivity C-reactive protein (hs-CRP), vascular endothelial growth factor A (VEGF-A), fibroblast growth factor-23 (FGF-23), regulated upon activation, normal T cell expressed and presumably secreted (RANTES), MIG, CRP, kidney injury molecule-1 (KIM-1) and homocysteine gradually increased with decreasing GFR. According to the results of univariate linear regression analysis, there were significant relationships between the levels of VEGF-A, FGF-23, RANTES, tumor necrosis factor alpha (TNF-alpha), MIG, CRP, hs-CRP, IL-6 and renal function. By multiple linear regression analysis adjusted for confounding factors, serum creatinine was significantly correlated with FGF-23 (β=0.40, P<0.001) and Il-6 (β=0.29, P<0.001).

Conclusion: Our study demonstrated the important role of growth factors and proinflammatory cytokines in the development and progression of CKD in DM. However, despite these data, the pathogenesis of diabetic changes in the kidneys remains not fully understood and, in order to clarify the possibility of pathogenetic influences on the progression of diabetic nephropathy, it is necessary to study various aspects of its development, including genetic factors.

Implication for health policy/practice/research/medical education:
Serum fibroblast growth factor-23 and interleukin-6 may play an important role of growth factors and proinflammatory cytokines in the development and progression of chronic kidney disease in diabetes mellitus.

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Original

Introduction

Diabetes mellitus (DM) and chronic kidney disease (CKD) are two chronic non-communicable diseases that have exceeded epidemic thresholds in all countries of the world. The problem of early diagnosis, prevention and treatment of CKD continues to be relevant for modern medicine, and assessment of the severity of CKD and associated cardiovascular complications is of great practical importance for primary and secondary prevention (1).

It is well known that one of the most important markers associated with kidney damage and the progression of CKD is persistent proteinuria (2). However, the presence of proteinuria already indicates the irreversibility of the pathological process in the kidneys. Another important marker that allows for diagnosis of preclinical nephropathy is microalbuminuria (3). As previous studies have shown, microalbuminuria is not the earliest and not the most specific marker of developing renal impairments in DM; it can appear in conditions not associated with diabetic nephropathy such as heavy physical exercises, a high-protein diet, fever, urinary infection, prolonged arterial hypertension, and congestive heart failure. In addition, albuminuria may not be detected in patients with tubulointerstitial kidney disease (4). Furthermore, approximately 30% of patients with diabetic nephropathy have normal urine albumin levels (5).

In clinical practice, to diagnose impaired renal function, serum creatinine and cystatin C levels are assessed, with the calculation of glomerular filtration rate (GFR), and the determination of albuminuria. Unfortunately, these markers are not always accurate. There is a non-linear correlation between creatinine and cystatin C levels and the GFR, and, therefore, a relatively small increase in these markers may indicate a significant decrease in the GFR (6-8). Because of some limitations of these markers, new alternative markers are being searched (9). Despite the high prevalence of CKD and the high cardiovascular risk associated with it, it often remains undiagnosed, and patients are not even aware that they have this pathology.

Currently, there are blood and urine markers that are increasingly being used to diagnose CKD in the early stage and therefore to prescribe appropriate nephroprotective therapy and reduce the need for renal replacement therapy. Candidate biomarkers include growth factors, cytokines, and chemokines. These molecules characterize damage to the renal glomeruli and tubules or indicate the development of oxidative stress and inflammation in the interstitial tissue of the kidneys (10).

Objectives

The aim of this study was to assess the role of proinflammatory cytokines, chemokines and growth factors in progression of CKD in patients with DM.

Patients and Methods

Study participants

We screened 155 patients with type 2 DM who consecutively visited the Republican Research Center for Radiation Medicine and Human Ecology (Gomel, Republic of Belarus). The inclusion criteria were as follows; a written informed consent, the presence of type 2 DM, age between 40 and 75 years, and body mass index (BMI) between 18.5 and 40.0 kg/m^2. All patients were treated with oral antidiabetic drugs and/or insulin. Patients with overt infection, thyroid gland disorders, accompanied by a manifest dysfunction, liver failure, active hepatitis, liver cirrhosis, systemic autoimmune diseases, diseases of the hematopoietic system, including moderate and severe anemia, primary renal pathology of non-diabetic origin, treated with glucocorticoids, immunosuppressants, immunomodulators, biological agents were excluded. Control group included 21 healthy individuals of the same age. Demographic parameters were evaluated, height and weight were measured, and BMI was calculated.

Biochemical measurements

Fasting blood samples were collected from each patient in the morning after overnight fasting. Serum was separated by centrifugation at 3000 rpm for 15 minutes within 2 hours after sample collection.

Hemoglobin A1c (HbA1c) was assayed using high-performance liquid chromatography. The level of homocysteine was measured by enzyme-linked immunosorbent assay (ELISA) using "Axis-Shield Diagnostics Limited" (England). Serum levels of C-reactive protein (CRP) and high-sensitivity CRP (hs-CRP) were determined by immunoturbidimetry on the biochemical analyzer "Architect c8000" (ABBOTT, USA, kits Biosystems S.A., Barcelona, Spain). Interleukin-6 (IL-6) was determined on an automatic laboratory analyzer Cobas 6000 for immunological and photometric tests Roche Diagnostics, (Germany) using original test systems.

The analysis of serum and urine biomarkers was carried out by enzyme immunoassay using commercial test systems:

- Serum fibroblast growth factor-23 (FGF-23) (C-Terminal) (Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria). Sensitivity: LOD: (0 pmol/L + 3SD): 0.08 pmol/L; LLOQ: 0.1 pmol/L. Specificity: This kit recognizes endogenous and recombinant human FGF-23 and measures both intact and C-terminal fragments of FGF-23. Assay variability scores: Intra-assay (n=6) ≤12%, Inter-assay (n=10) ≤ 10%.
- Tumour necrosis factor alpha (TNF-alpha) (Human TNF alpha Platinum ELISA, eBioscience, Vienna, Austria). Sensitivity: 2.3 pg/mL. Intra-assay – 6.0%, Inter-assay – 7.4%.

- Monokine induced by gamma interferon (MIG) (Human MIG Instant ELISA, eBioscience, Vienna, Austria). Sensitivity: 4 pg/mL. Intra-assay – 5.4%, Inter-assay – 11%.
- Vascular endothelial growth factor A (VEGF-A) (Human VEGF-A Platinum ELISA, eBioscience, Vienna, Austria). Sensitivity: 7.9 pg/mL. Intra-assay - 6.2%, Inter-assay – 4.3%.
- Regulated upon activation, normal T cell expressed and presumably secreted (RANTES) (Human RANTES Instant ELISA, eBioscience, Vienna, Austria). Sensitivity: 4.2 pg/mL. Intra-assay - 6.9%, Inter-assay – 9.9%.
- Kidney injury molecule-1 (KIM-1) in urine (human) Elisa Kit (Enzo Life Sciences (ELS) AG, Switzerland). Sensitivity: 1.279 pg/mL. Specificity: ≤0.02%. Intra-assay 385.5 pg/mL – 1.8% CV, 93.1 pg/mL – 2.3% CV, 39.3 pg/mL – 2.6% CV, Inter-assay 397.5 pg/mL ml - 6.2% CV, 99.8 pg/mL – 6.4% CV, 39.7 pg/mL – 1.9% CV.

Renal function was assessed based on the levels of serum creatinine; cystatin C. GFR was calculated according to the CKD-EPI (chronic kidney disease epidemiology collaboration) equation (11). Urinary albumin creatinine ratio (A/C) was determined from urinary creatinine and albumin measurements from spot midstream urine.

Statistical analysis

Data are presented as median (25^{th}-75^{th} percentile) where appropriate. Spearman's rank correlation analysis was performed to evaluate associations of studied markers. For bivariate comparisons the Kruskal-Wallis test was conducted. Multivariate linear regression analysis was performed to evaluate FGF-23 and IL-6 as potential independent predictors of CKD. *P* value <0.05 was considered statistically significant. Statistical processing of the obtained data was performed using Stata version 14.2 software (StataCorp, Texas, USA).

Results

Characteristics of the study participants are shown in Table 1. DM patients had significantly higher levels of proinflammatory cytokines in comparison with the control group (TNF-alpha – 12.42 (7.80-20.00) versus 9.80 (3.89-11.20) pg/mL, IL-6 – 3.14 (1.70-9.10) versus 1.50 (1.50-1.70) mg/mL), chemokines (RANTES – 82.99 (65.60-120.21) versus 60.30 (52.75-69.10) ng/mL, MIG – 167.50 (89.70-675.60) versus 95.42 (59.80-114.30) pg/mL) and growth factors (VEGF-A – 365.28 (233.58-765.50) versus 237.30 (149.20-305.40) pg/mL, FGF-23 – 1.09 (0.39-6.04) versus 0.57 (0.19-2.19) pmol/l). The levels of CRP and hs-CRP were significantly higher in patients with DM (5.20 (2.10-8.50) and 6.30 (3.10-10.10) mg/L versus 1.10 (0.90-1.60) and 1.90 (1.20-2.70) mg/L in control group, respectively). The levels of KIM-1 and homocysteine were 566.70 (289.16-975.84) pg/mL and 11.20 (8.00-16.50) µmol/L in DM patients, while in the control group they were 122.30 (102.10-245.30) pg/mL and 7.30 (6.70-8.30) µmol/L; $P<0.001$, respectively. We divided the patients into five groups depending on the stages of CKD (Table 2).

Table 1. Baseline characteristics of the study subjects

Variables	DM group, n=155	Control group, n=21	*P*
Age, years	65.00 (55.00-71.00)	63.00 (36.00-64.00)	0.115
BMI, kg/m^2	29.69 (25.22-34.95)	30.06 (23.74-32.87)	0.552
Creatinine, mmol/l	100.00 (71.00-264.00)	60.00 (55.00-76.00)	<0.001
HbA1c, %	8.70 (7.90-9.50)	4.80 (4.30-5.10)	<0.001
A/C, mg/mmol	8.60 (2.60-27.50)	0.85 (0.70-1.40)	<0.001
VEGF-A, pg/mL	365.28 (233.58-765.50)	237.30 (149.20-305.40)	<0.001
FGF-23, pmol/L	1.09 (0.39-6.04)	0.57 (0.19-2.19)	0.034
RANTES, ng/mL	82.99 (65.60-120.21)	60.30 (52.75-69.10)	<0.001
TNF-alpha, pg/mL	12.42 (7.80-20.00)	9.80 (3.89-11.20)	0.001
MIG, pg/mL	167.50 (89.70-675,60)	95.42 (59.80-114.30)	<0.001
CRP, mg/L	5.20 (2.10-8.50)	1.10 (0.90-1.60)	<0.001
hs-CRP, mg/L	6.30 (3.10-10.10)	1.90 (1.20-2.70)	<0.001
IL-6, mg/mL	3.14 (1.70-9.10)	1.50 (1.50-1.70)	<0.001
KIM-1, pg/mL	566.7 (289.16-975.84)	122.30 (102.10-245.30)	<0.001
Homocysteine, µmol/L	11.2 (8.0-16.5)	7.30 (6.70-8.30)	<0.001

BMI, Body mass index; HbA1c, Hemoglobin A1c; A/C, Urinary albumin-creatinine ratio; VEGF-A, Vascular endothelial growth factor A; FGF-23, Fibroblast growth factor-23; RANTES, Regulated upon activation normal t cell expressed and presumably secreted; TNF-alpha, Tumour necrosis factor alpha; MIG, Monokine induced by gamma interferon; CRP, C-reactive protein; hs-CRP, high-sensitivity C-reactive protein; IL-6, Interleukin-6; KIM-1, Kidney injury molecule-1.
Note: Values are median (25th–75th percentile).

The level of IL-6 significantly increased as GFR decreased; from 1.70 (1.50-1.90) mg/mL in CKD stage one to 11.40 (8.90-32.10) mg/mL in CKD stage five. At the same time, IL-6 values in patients with CKD stage one was also significantly higher compared to the control group (1.70 (1.50-1.90) mg/mL versus 1.50 (1.50-1.70; $P < 0.001$).

The same results were obtained for hs-CRP: the level of this marker was higher in patients with CKD stage one compared to the control group (3.20 (2.40-5.00) mg/L versus 1.90 (1.20-2.70) mg/L, respectively) and increased with a decrease in GFR to 11.25 (7.80-14.30) mg/L. However, there were no significant differences between patients with CKD stages 4 and 5 in terms of the level of hs-CRP.

In addition to IL-6 and hs-CRP, the level of VEGF-A was significantly higher in patients with DM in the CKD stage one group compared to the control group (300.00 (226.60-441.66) pg/mL versus 237.30 (149.20-305.40) pg/mL). The highest values of this marker (as well as the levels of FGF-23, RANTES, MIG, CRP, KIM-1 and homocysteine) were observed in DM patients with CKD 5 (718.75 (342.10-894.30) pg/mL).

According to the results of univariate linear regression analysis, there were significant relationships between the levels of VEGF-A, FGF-23, RANTES, TNF-alpha, MIG, CRP, hs-CRP, IL-6 and renal function (Table 3).

The building of correlation networks with the establishment of a threshold value of the correlation coefficient (moderate and strong correlation) made it possible to identify the relationship of the studied markers with the main parameters of renal function (Figure 1). When the threshold value of the correlation coefficient was set to >0.7, it was found that there were no correlations between VEGF-A, RANTES, TNF-alpha, MIG, KIM-1 in relation to other markers (Figure 2).

By multiple linear regression analysis adjusted for

Table 2. Characteristics of the study subjects depending on the CKD stages

Parameter	DM group, n=155					Control group (n=21)	*P*
	CKD 1 (n=35)	CKD 2 (n=30)	CKD 3 (n=25)	CKD 4 (n=27)	CKD 5 (n=38)		
VEGF-A, pg/mL	300.00[a] (226.60-441.66)	339.24[a] (220.80-567.02)	332.64[a] (233.58-657.44)	465.50[a,b] (187.40-835.40)	718.75[a,b,c,d] (342.10-894.30)	237.30 (149.20-305.40)	0.0001
FGF-23, pmol/L	0.40 (0.19-0.86)	0.39[a] (0.17-1.25)	0.75[a] (0.38-1.04)	6.50[a,b,c,d] (1.54-9.67)	7.29[a,b,c,d] (3.22-10.95)	0.57 (0.19-2.19)	0.0001
RANTES, ng/mL	69.66 (50.26-83.68)	73.93[a] (63.59-84.96)	77.50[a] (67.01-97.86)	110.40[a,b,c,d] (73.20-150.10)	120.25[a,b,c,d] (84.50-157.30)	60.30 (52.75-69.10)	0.0001
TNF-alpha, pg/mL	10.46 (6.02-15.20)	13.18[a] (6.52-19.58)	11.20[a] (5.44-15.74)	14.90[a] (7.40-29.10)	15.32[a,b] (12.30-32.40)	9.80 (3.89-11.20)	0.0001
MIG, pg/mL	95.72 (62.40-125.00)	100.72[ad] (70.24-188.46)	165.5[a,b] (116.08-204.3)	457.40[a,b,c,d] (233.10-943.40)	731.65[a,b,c,d] (344.20-2009.50)	95.42 (59.80-114.30)	0.0001
CRP, mg/L	1.90 (1.30-3.70)	2.60[a] (1.60-4.30)	5.80[a,b] (3.40-8.70)	6.90[a,b,c] (5.37-8.30)	8.80[a,b,c,d] (6.20-11.50)	1.10 (0.90-1.60)	0.0001
hs-CRP, mg/L	3.20[a] (2.40-5.00)	3.10[ad] (2.40-5.10)	6.30[a,b] (4.20-11.20)	8.60[a,b,c] (6.51-10.10)	11.25[a,b,c] (7.80-14.30)	1.90 (1.20-2.70)	0.0001
IL-6, mg/mL	1.70[a] (1.50-1.90)	2.22[ad] (1.70-3.20)	3.20[a,b] (2.30-8.70)	3.40[a,b,c] (1.80-8.60)	11.40[a,b,c,de] (8.90-32.10)	1.50 (1.50-1.70)	0.0001
KIM-1, pg/mL	452.31[a] (197.11-944.12)	512.84[a] (265.08-915.40)	478.92[a] (317.76-765.70)	654.30[a] (267.7-130.90)	783.75[a,b,d] (462.64-1334.30)	122.30 (102.10-245.30)	0.0001
Homocysteine, µmol/L	7.65 (6.90-8.90)	10.32[a,b] (8.30-12.70)	11.50[a,b] (10.50-16.50)	14.60[a,b,c] (8.50-18.30)	18.70[a,b,c,de] (11.20-26.50)	7.30 (6.70-8.30)	0.0001
Creatinine, nmol/l	61.00 (55.00-69.00)	74.00[a,b] (69.00-84.00)	100.00[a,b,c] (94.00-128.00)	200.00[a,b,c,d] (179.00-223.00)	430.50[a,b,c,de] (320.00-529.00)	60.00 (55.00-76.00)	0.0001
Cystatin C, mg/L	0.74 (0.62-0.83)	0.91[a,b] (0.78-0.98)	1.28[a,b,c] (1.12-1.78)	2.56[a,b,c,d] (1.99-2.99)	3.68[a,b,c,de] (3.14-4.10)	0.61 (0.58-1.15)	0.0001
Proteinuria, g/24h	0.03[a] (0.01-0.05)	0.05[a] (0.02-0.07)	0.30[a,b,c] (0.05-0.90)	0.87[a,b,c,d] (0.36-1.13)	1.05[a,b,c,de] (0.87-1.54)	0	0.0001
A/C, mg/mmol	2.60[a] (0.72-6.70)	2.70[a] (2.33-5.40)	8.27[a,b,c] (3.64-25.40)	21.30[a,b,c,d] (12.50-32.82)	29.60[a,b,c,de] (18.70-113.40)	0.85 (0.70-1.40)	0.0001

VEGF-A, Vascular endothelial growth factor A; FGF-23, Fibroblast growth factor-23; RANTES, Regulated upon activation normal T cell expressed and presumably Secreted; TNF-alpha, Tumour necrosis factor alpha; MIG, Monokine induced by gamma interferon; CRP, C-reactive protein; hs-CRP, high-sensitivity C-reactive protein; IL-6, Interleukin-6; KIM-1, Kidney injury molecule-1; A/C, Urinary albumin-creatinine ratio.

[a] $P < 0.001$ in comparison with the control group, [b] $P < 0.05$ in comparison with the CKD 1 group, [c] $P < 0.01$ in comparison with the CKD 2 group, [d] $P < 0.05$ in comparison with the CKD 3 group, [e] $P < 0,05$ in comparison with the CKD 4 group.

Note: Values are median (25th–75th percentile).

Table 3. Univariate correlations between markers of kidney function and other variables

	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
	IL-6		**FGF-23**		**CRP**		**hs-CRP**	
Creatinine	0.70	<0.001	0.71	<0.001	0.56	<0.001	0.59	<0.001
GFR	-0.73	<0.001	-0.69	<0.001	-0.58	<0.001	-0.61	<0.001
Cystatin C	0.71	<0.001	0.65	<0.001	0.61	<0.001	0.66	<0.001
Proteinuria	0.50	<0.001	0.52	<0.001	0.49	<0.001	0.54	<0.001
A/C	0.44	<0.001	0.52	<0.001	0.52	<0.001	0.57	<0.001
	VEGF-A		**RANTES**		**TNF-alpha**		**MIG**	
Creatinine	0.31	0.001	0.49	<0.001	0.36	<0.001	0.55	<0.001
GFR	-0.33	<0.001	-0.51	<0.001	-0.36	<0.001	-0.57	<0.001
Cystatin C	0.35	<0.001	0.48	<0.001	0.37	<0.001	0.57	<0.001
Proteinuria	0.26	0.0012	0.40	<0.001	0.17	0.04	0.50	<0.001
A/C	0.19	0.018	0.31	0.0001	0.32	0.0001	0.44	<0.001
	Homocysteine				**KIM-1**			
	r	*P*			*r*	*P*		
Creatinine	0.58	<0.001			0.20	0.013		
GFR	-0.59	<0.001			-0.22	0.004		
Cystatin C	0.58	<0.001			0.20	0.012		
Proteinuria	0.47	<0.001			0.17	0.039		
A/C	0.34	<0.001			0.21	0.009		

GFR, Glomerular filtration rate; VEGF-A, Vascular endothelial growth factor A; FGF-23, Fibroblast growth factor-23; RANTES, Regulated upon activation normal t cell expressed and presumably secreted; TNF-alpha, Tumour necrosis factor alpha; MIG, Monokine induced by gamma interferon; CRP, C-reactive protein; hs-CRP, high-sensitivity C-reactive protein; IL-6, Interleukin-6; KIM-1, Kidney injury molecule-1; A/C, Urinary albumin-creatinine ratio.

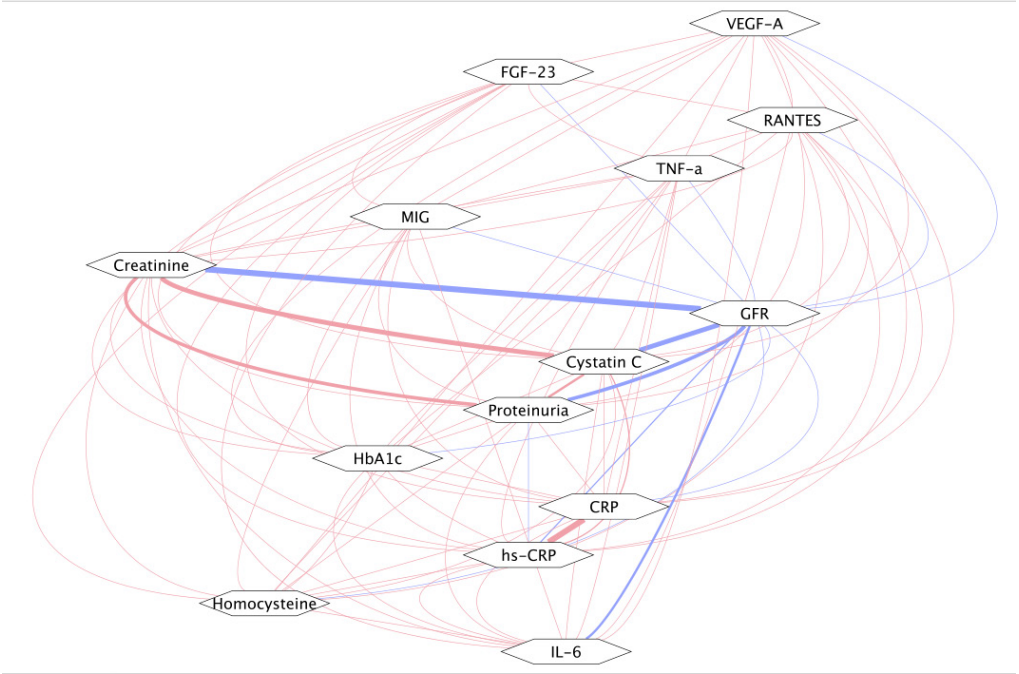

Figure 1. Schematic representation of the correlation network based on cytokines, chemokines and growth factors concentrations in patients with type 2 diabetes (the thickness of the lines represents the strength of the correlation. Red lines reflect positive correlations. Blue lines reflect negative correlations).

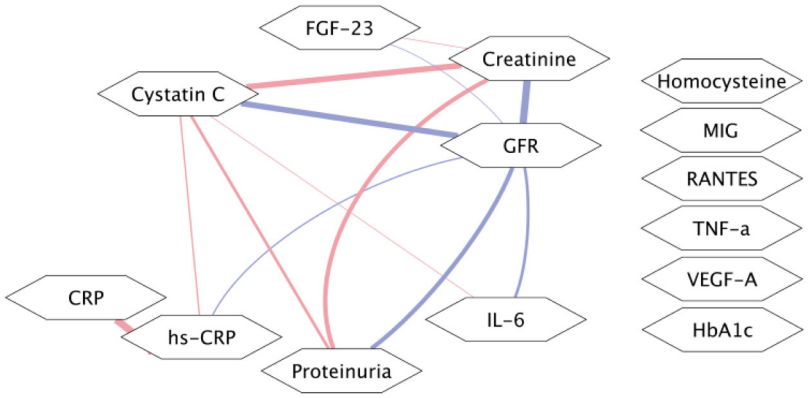

Figure 2. Schematic representation of the correlation network based on cytokines, chemokines and growth factors concentrations calculated at the threshold of >0.7 correlation coefficient in patients with diabetes type 2 (The thickness of the lines represents the strength of the correlation. Red lines reflect positive correlations. Blue lines reflect negative correlations).

confounding factors, creatinine was significantly correlated with FGF-23 ($\beta = 0.40$, $P<0.001$) and IL-6 ($\beta = 0.29$, $P<0.001$; Table 4).

Discussion

The causes for changes in cytokine production in DM are unclear. The role of hyperglycemia and insulin deficiency as factors modifying cytokine production is still discussed. It is assumed that hyperglycemia induces oxidative stress, activates the action of growth factors, vasoactive factors, cytokines, causing damage to the kidneys at the cell level. This leads to the development of renal hypertrophy and accumulation of the extracellular matrix, preceding such irreversible changes as glomerulosclerosis and tubulointerstitial fibrosis (12,13).

Table 4. Multivariate linear regression analysis for putative predictors of CKD

Variables	β	*P*	95% CI
Age	-0.47	0.46	-1.72-0.79
Homocysteine	1.41	0.11	-0.34-3.16
hs-CRP	1.10	0.38	-1.35-3.55
VEGF-A	0.06	0.05	-0.00-0.12
FGF-23	9.69	<0.001	3.30-16.09
RANTES	0.32	0.20	-0.17-0.81
TNF-alpha	0.29	0.74	-1.44-2.02
MIG	-0.01	0.57	-0.03-0.02
IL-6	3.99	<0.001	2.20-5.78
KIM-1	-0.02	0.41	-0.05-0.02

$R^2 = 0.67$

All variants are adjusted for the analysis.

β: standardized regression coefficient; CI: confidence intervalhs-CRP, high-sensitivity C-reactive protein; VEGF-A, Vascular endothelial growth factor A; FGF-23, Fibroblast growth factor-23; RANTES, Regulated upon activation normal T cell expressed and presumably secreted; TNF-alpha, Tumour necrosis factor alpha; MIG, Monokine induced by gamma interferon; IL-6, Interleukin-6; KIM-1, Kidney injury molecule-1.

In recent years, many studies have been devoted to unveiling the molecular mechanisms involved in inflammation-mediated kidney damage. It has been shown that circulating levels of the proinflammatory cytokine IL-6 are elevated in CKD and in dialysis patients (14,15). In our study, we found that patients with CKD showed a statistically significant increase in the level of IL-6 with the decrease in GFR. We were able to demonstrate a strong significant relationship between eGFR, creatinine, cystatin, and serum IL-6 levels. This relationship was preserved in the conducted multiple regression analysis ($\beta = 3.99$, $P<0.001$), even despite the influence of other markers.

Previously, Gutiérrez et al (16) were the first who demonstrated a strong association between serum FGF-23 levels and mortality among “dialysis” patients. Later these associations were confirmed in patients with CKD who are not on dialysis, and even in individuals with normal kidney function (17,18). The fact that a high level of FGF-23 is a clinically significant risk factor for the development of severe cardiovascular complications is noted in almost all studies on this issue.

The mechanism of FGF-23 influence on the progression of CKD is still not completely clear. On the one hand, under the influence of FGF-23, renal phosphorus excretion is stimulated due to direct suppression of sodium phosphate co-transporters in the proximal tubules (19). As kidney function deteriorates, FGF-23 levels progressively increase. This is an adaptive process aimed at maintaining the balance of phosphate in the body in conditions of a decrease in the ability of the kidneys to excrete it in CKD (20). Maintaining normal blood phosphate levels is clearly beneficial, as hyperphosphatemia or even moderate elevations of phosphate levels within reference ranges are known to be a risk factor for poor renal outcomes (21). On the other hand, Fliser et al (22) found that FGF-23, rather than serum phosphorus, is an important

independent predictor of CKD progression, explaining that in patients with end-stage renal disease, serum FGF-23 levels are significantly elevated in response to chronic phosphorus overload and active vitamin D therapy. As a result, an increased level of FGF-23 cannot compensate for the retention of phosphorus and a decrease in the mass of active nephrons limits the ability of the kidneys to excrete phosphorus. In our present study, we demonstrated high levels of FGF-23 in patients with DM compared with those without DM. FGF-23 levels increased as renal function declined; from 0.40 (0.19-0.86) pmol/L to 7.29 (3.22-10.95) pmol/L. We found a strong association of FGF-23 and creatinine, demonstrated both in the correlation analysis and preserved in the multiple regression analysis ($\beta = 9.69$, $P < 0.001$).

Despite the fact that in this study, other investigated markers have lost their relationship with the level of creatinine, they play an important role in the activation of pathological processes during the progression of nephropathy (23,24). For example, overexpression of TNF-alpha has a cytotoxic, immunomodulatory, proinflammatory effect, activating oxidative stress. TNF-alpha regulates the proliferation of mesangial cells, the synthesis of the extracellular matrix, which leads to the development of fibroplastic and sclerotic processes in the kidneys. In this study, there were no significant difference between the levels of TNF-alpha in patients with DM and CKD stage one and control group, but starting from the stage 2 of CKD, the level of the cytokine gradually increased with maximum values in CKD 5 (10.46 (6.02-15.20) pg/mL). Analysis of TNF-alpha correlations showed a moderate relationship with creatinine, cystatin C, eGFR, KIM-1, and proteinuria.

DM is characterized by significant changes in the synthesis of angiogenesis regulators in the kidneys. It has been shown that the glomerular neovascularization in patients with DM correlates with VEGF expression (25). In this study, the level of VEGF-A was significantly higher in patients with DM compared with the control group, and significantly differed depending on the stage of CKD. However, there were weak positive correlations with the level of proteinuria (r=0.26, $P = 0.0012$), creatinine level (r=0.31, $P = 0.001$), cystatin C (r=0.35, $P < 0.001$), and there was no significant association with creatinine in multiple linear regression analysis (β=0.06, $P = 0.05$).

Tubulointerstitial renal fibrosis is just as important as a mechanism for the loss of kidney function in DM as is glomerulosclerosis. In the development of tubulointerstitial damage, the role of complex processes of intercellular interactions, which are activated under the influence of immune and non-immune factors, is determined, and acute phase proteins CRP and hs-CRP are distinguished among the possible factors of tubulointerstitial damage. There is an opinion that the serum level of CRP is extremely nonspecific and has weak correlations with the main signs of kidney damage in DM. However, in population studies it has been shown that a decrease in GFR is associated with an increase in the concentration of CRP. This was demonstrated in the present study, where high values of these proteins were detected in patients with CKD 5 (8.80 (6.20-11.50) mg/L and 11.25 (7.80-14.30) mg/L versus 1.90 (1.30-3.70) mg/L and 3.20 (2.40-5.00) in CKD 1, respectively).

One of the factors that significantly impairs the kidneys and increases the risk of early development of cardiovascular complications is homocysteine. Significantly higher homocysteine levels were demonstrated in patients with DM and CKD compared with the control group (566.70 (289.16-975.84) μmol/L versus 122.30 (102.10-245.30) μmol/L, $P < 0.001$, respectively).

Conclusion

Our study demonstrated the important role of growth factors and proinflammatory cytokines in the development and progression of CKD in DM. However, despite these data, the pathogenesis of diabetic changes in the kidneys remains not fully understood and, in order to clarify the possibility of pathogenetic influences on the progression of diabetic nephropathy, it is necessary to study various aspects of its development, including genetic factors.

Limitations of the study

Several limitations need to be addressed in the present study. First, our sample size was relatively small. We did not assess α-klotho levels, which should be considered for a proper interpretation of the effects elicited by FGF23. We did not have information on the use of vitamin D or calcium supplements, or measures of phosphate, parathyroid hormone or calcium levels, which might potentially interact on the FGF-23 concentration. Owing to the observational nature of this study, the associations cannot be interpreted as causal and residual confounding cannot be excluded.

Authors' contribution

Conceptualization: IS.
Methodology: VB, IS and NK.
Validation: IS and NK.
Formal analysis: VV, IS.
Investigation: IP and VV.
Resources: VB, IS and VV.
Data curation: TM.
Writing–original draft preparation: VV and IP.
Writing–review and editing: TM, VV and IP.
Visualization: VB.
Supervision: TM.
Project administration: VV and IP.

Conflicts of interest

All authors declare that they do not have a conflict of

interest with the contents of this paper.

Ethical issues

Human rights were respected in accordance with the Helsinki Declaration 1975, as revised in 1983. Informed consent was obtained from all patients to conduct this study. The ethical committee of The Republican Research Center for Radiation Medicine and Human Ecology approved this study (Registration Number 134 M 6542). Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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