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Cardiovascular Features of Chronic Inflammation and Endothelial Dysfunction in Patients with Diabetic Nephropathy on Programmed Hemodialysis

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Abstract

Introduction and purpose: It is important today to determine the factors that form a very high cardiovascular risk in patients with type 2 diabetes mellitus (DM) with kidney damage on programmed hemodialysis (HD). The aim of the study has been to determine the cardiovascular features of chronic inflammation and endothelial dysfunction (ED) in HD patients with diabetic nephropathy (DN). **Material and methods:** The study has included 136 patients treated with HD (men, 78; age, 53.9±1.0 year, duration of HD, 47.6±4.2 months). Depending on the presence/absence of type 2 diabetes, they have been divided into two groups: the first – without DN (n=88); the second – with DN (n=48). The intensity of inflammation has been assessed by the serum content of tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP), fibrinogen (FG) and albumin. Vasomotor function of the brachial artery (BA) has been determined using a test with reactive hyperemia (endothelium-dependent vasodilation, (EDVD)). The content of nitrite anions (NO_2-) and the number of circulating endothelial cells (CECs) in blood plasma have been measured. **Results**: In patients with DN, TNF-α ($p=0.002$), CRP ($p<0.001$) and FG ($p=0.008$) significantly exceeded those in non-diabetics. The average value of EDVD BA ($p=0.001$), NO_2- ($p=0.008$) in patients of the second group has been lower, and the number of

CECs – higher ($p<0.001$) compared with the first group. Vasoconstriction (EDVD<0%) and undilatation (EDVD=0%) reactions in the case of DN have been registered more often (52.1 vs. 19.1%, $p<0.001$). In patients with DN for the first time have been established correlations between CECs and TNF-α ($Rs=0.73$, $p<0.001$), CRP ($Rs=0.53$, $p<0.001$), FG ($Rs=0.53$, $p<0.001$), albumin ($Rs=-0.43$, $p=0.002$). **Conclusions**: Chronic inflammation in the constellation with endothelial damage in patients with DN in the case of HD is obviously an important factor in cardiovascular remodeling, a predictor of progression of cardiovascular complications.

Key words: programmed hemodialysis; diabetic nephropathy; cardiovascular risk; proinflammatory mediators; endothelial damage; pathogenesis

Introduction

Numerous clinical studies have shown [1, 2] that diabetic nephropathy (DN) in patients with chronic kidney disease (CKD) is associated with a very high cardiovascular risk. The mechanisms of maladaptive myocardial remodeling, progression of atherosclerotic and arteriosclerotic damage, development of fatal and non-fatal cardiovascular events in patients with type 2 diabetes mellitus (DM) with kidney damage on programmed hemodialysis (DM) involve a set of factors related to uremic toxins, hypertension, dyslipidemia, disorders of mineral metabolism, hyperhydration, anemia, ectopic calcification, oxidative stress (OS) activity, chronic inflammation, insulin resistance and endothelial dysfunction (ED) [3, 4, 5, 6]. According to the analysis of literature, the role of inflammation as a non-traditional risk factor in the formation of cardiovascular disease in the case of DN and treatment of HD is insufficiently defined. Thus, reports on the dependence of type 2 diabetes mellitus with kidney damage with chronic inflammation in HD patients are contradictory and ambiguous [4, 5, 7, 8], the relationship between markers of chronic inflammatory activity and the structural and functional state of the endothelium remains unclear under these conditions. It is believed [9, 10] that in the case of CKD endothelial damage with alteration of nitric oxide (NO) metabolism is an important mechanism that mediates the effects of systemic manifestations of inflammation on the remodeling of cardiovascular system and a key component of cardiovascular complications.

Purpose

The aim of study – to determine the cardiovascular features of chronic inflammation and ED in patients with DN treated with programmed HD.

Material and methods

The observational cross-sectional study has included 136 patients with CKD VD stage, who have been treated from chronic HD, in the Hemodialysis Department of Ternopil University Hospital (Ukraine). There have been 78 men and 58 women. The mean age of patients has been 53.9±1.0 years, HD duration has been 47.6±4.2 months. There have been 35.3% of patients with DN, 32.4% with chronic glomerulonephritis, 14.0% with chronic pyelonephritis, 5.1% with polycystic kidney disease, 4.4% with hypertensive nephropathy, and 8.8% with other diseases.

The study has complied with patient safety rules, preserved their rights and canons of human dignity, as well as moral and ethical norms that comply with the basic provisions of the GSP (1996), the Council of Europe Convention on Human Rights and Biomedicine (1997), Helsinki Declaration of the World Medical Association on the ethical principles of conducting scientific medical research with human participation (1964–2008). All patients have given informed consent to participate in the study. The study protocol has been approved by the Commission on Bioethics of I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine. Criteria for exclusion of patients from the study has been type 1 diabetes, age<18 years, duration of treatment HD<6 months, eKt/V<1.4, acute and delayed (up to 6 months) myocardial infarction or stroke, haemoglobin level <80 g/L, obstructive lung disease, severe liver pathology, mental disorders, absence of consent to participate in the study.

According to the study design, depending on the presence/absence of type 2 DM with kidney damage, all HD-patients have been divided into two groups: the first – without DN (n=88); the second – with DN (n=48). The duration of DM in patients with DN has been 174.7±7.1 months. When conducting clinical-diagnostic and treatment measures, scientists have relied on the protocols of diagnosis and treatment, approved by the order of the Ministry of Health of Ukraine dated February 11, 2016 No. 89, on the recommendations of KDOQI and KDIGO on the diagnosis and treatment of CKD. HD patients have been performed according to the standard program (3 times a week for 4–4.5 h) using synthetic dialyzers and bicarbonate buffer. The provided dose of dialysis (Kt/V ratio) has been calculated by the formula of natural logarithm [11].

The intensity of inflammatory process has been assessed by the serum content of tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP), fibrinogen (FG) and albumin. The concentration of TNF-α has been determined by enzyme-linked immunosorbent assay using mono- and polyclonal antibodies to TNF-α using a set of reagents from the firm “Best” (Russia), CRP – immunoturbidimetric method, albumin – the end point of formation of a colored

complex with green medium, FG – unified gravimetric method. CRP and albumin concentrations have been tested on an automated computer analyzer Integra 400 Plus of the company "Roche" (Switzerland). Isolation and quantitative analysis of circulating endothelial cells (CECs) as an index of endothelial damage in platelet-enriched plasma has been performed according to the method [12]; NO production has been evaluated by the plasma content of its stable metabolite nitrite anion (NO_2-) by Green spectrophotometric method using Griess reagent [13].

Vasomotor function of the brachial artery (BA) has been determined by duplex ultrasound scanning using a test with reactive hyperemia (RH) on a scanner "HDI 1500-Philips" (USA) using a sensor with a frequency of 7.5 MHz [14]. Changes in vessel diameter in 60 s after decompression (maximum artery dilatation time) in the sample with RH (endothelium-dependent vasodilation (EDVD)) have been evaluated as a percentage of baseline. The norm has been an increase in diameter BA of 10% or more. Less importance of dilatation, vasoconstriction or absence of dynamics of EDVD indices has been regarded as a pathological reaction.

STATISTICA® Version 10.0 software package from the company "StatSoft, Inc." (USA) has been used for statistical data analysis. Methods of nonparametric statistics have been used – Mann-Whitney U-test to compare quantitative indices in two independent groups, Pearson's χ^2-test to compare frequency values, Spearman's rank correlations (Rs) to determine the presence, direction and strength of relationship between the studied indices. When describing quantitative variables, the average values and their standard errors (M±m), qualitative binary – percent (%) have been given. Differences at $p<0.05$ have been considered statistically significant.

Results

The results of study of chronic inflammation activity in groups of HD patients with type 2 DM with kidney damage are shown in Table 1. It has been found that in patients with DN indicators of TNF-α, CRP and FG significantly exceeded those in non-diabetic patients. 58.8, 40.6 and 14.4%, respectively, while the level of albumin in the first and second groups has been similar.

Table 1. Indices of activity of chronic inflammation in patients with CKD VD stage, which differ DN (M±m)

Indicator	DN		Z	p
	absent (n=88)	present (n=48)		
TNF-α [ng/L]	8.73±0.60	13.86±1.34	3.04	0,002
CRP [mg/L]	7.07±1.09	9.94±1.12	3.47	<0.001
FG [g/L]	4.71±0.15	5.39±0.23	2.64	0.008
Albumin [g/L]	41.8±0.6	39.5±1.0	1.78	0.076

According to the results of Celermajer-Sorensen test, significant disorders of vascular function of the endothelium in patients with CKD VD stage, who have been treated with programmed HD; the flow-mediated dilatation of BA as a whole on group has made 4.12±0.42%. Thus, only 17.6% of HD patients observed adequate vasodilation (EDVD≥10%), and 82.4% – ED. Among patients with impaired vasodilation response, 16.9% had paradoxical vasoconstriction (EDVD<0%), 14.0% had no dynamics of EDVD during the RH phase (EDVD=0%), and 51.5% had insufficient vasodilation (0.1–9.9%) (Fig. 1). Characteristically, vasoconstriction and undilatation reactions of the vascular endothelium in patients with DN have been more common (52.1 vs. 19.1%; χ^2=15.6, p<0.001), while normal BA reactions have been less common (8.3 vs. 22.7%; χ^2=4.4, p= 0.035) than in patients without DM.

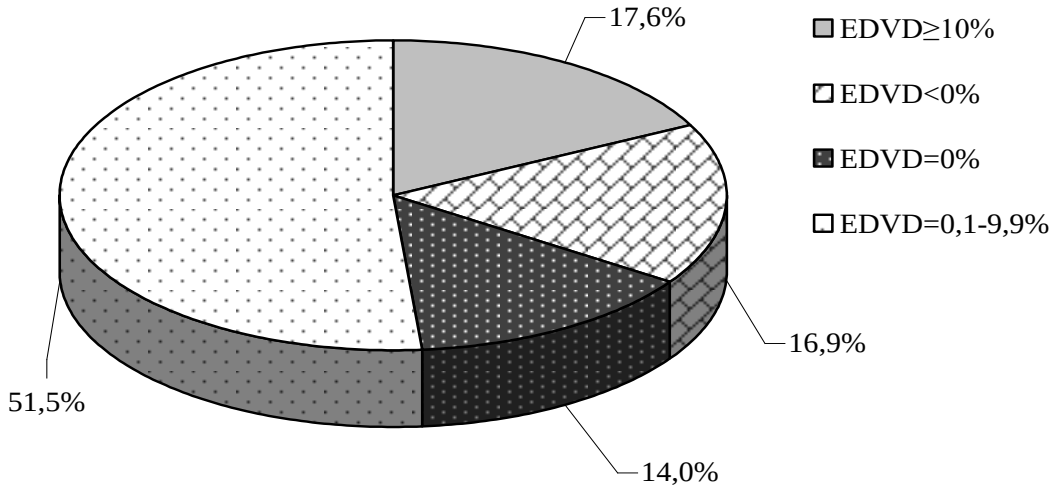

Fig. 1. Types of EDVD BA (test with RH) in patients with CKD VD stage

The data presented in Table 2 show a clear relationship between DN and ED in the case of programmed HD, which was confirmed by the worse dynamics of structural and functional parameters of the endothelium in the second group relative to the first. Thus, the average value

of EDVD BA (in 2.26 times), the content of NO_2- (in 2.17 times) in patients with type 2 DM with kidney damage has been significantly lower, and the number of CECs, on the contrary, higher (in 1.57 times) compared with patients without diabetes.

Table 2. Indices of ED in patients with CKD VD stage, which differ DN (M±m)

Indicator	DN		Z	p
	absent (n=88)	present (n=48)		
EDVD [%]	5.13±0.52	2.27±0.66	3.26	0.001
NO_2- [μmol/L]	9.01±1.37	4.16±0.41	3.15	0.002
CECs [$\times 10^4$/L]	14.2±0.7	22.3±1.3	4.98	<0.001

We showed for the first time a significant correlation between proinflammatory markers (mostly TNF-α and CRP) and ED values in patients with DN treated with HD (Table 3).

Table 3. Correlation model of chronic inflammation and ED activity in the group of HD patients with DN

	EDVD	NO_2-	CECs
TNF-α	Rs=-0.54, p<0.001	Rs=-0.61, p<0.001	Rs=0.73, p<0.001
CRP	Rs=-0.39, p=0.006	Rs=-0.43, p=0.003	Rs=0.53, p<0.001
FG	Rs=-0.29, p=0.043	Rs=-0.30, p=0.040	Rs=0.53, p<0.001
Albumin	Rs=0.44, p=0.002	Rs=0.28, p=0.056	Rs=-0.43, p=0.002

Discussion

Finding out the reasons for the development and progression of cardiovascular disorders in the case of DN is important not only to determine the role of CKD in the cardiovascular continuum, but also to develop new approaches to modern preventive nephrology. Today in the solution of the problem of ultrahigh frequency of coronary catastrophes, cardiac arrhythmias, cerebrovascular complications, congestive heart failure and sudden death in patients with type 2 diabetes with renal impairment in HD more and more importance is given to the study of chronic inflammation and ED [15, 16], because proinflammatory mediators and damaged endothelial cells are not only markers of DN progression with a negative impact on the prognosis, but also independent factors of increased cardiovascular risk [17, 18, 19].

In this study, DN in patients with CKD VD stage is combined with the activation of systemic manifestations of inflammation – the accumulation of CRP, TNF-α, FG, which echoes the work of many authors [7,8,20] who observed cytokine aggression in case of DM and programmed HD. At the same time Hsu et al. [5] do not find differences in the intensity of inflammatory process between groups of HD patients with and without DN, and Avci et al. [4] report on

inhibition of cellular inflammatory activity against OS under these conditions. Decreased serum albumin levels in patients treated with HD is demonstrated in a study of Jakuszewski et al. [8].

High level of TNF-α as one of the most important proinflammatory and angiogenic factors [16] in HD patients with DN, apparently, is a consequence of activation of macrophages, neutrophils, eosinophils, endothelial cells [21]. In addition, the cytokine profile with T-cell immunity in case of type 2 DM with kidney damage and CKD VD stage may depend on HD-associated factors [22], residual renal function, autonomic nervous system activity and other risks [5]. TNF-α stimulates the secretion of prostaglandins, has a chemotactic effect on various cells, activates transcription factors, causes the synthesis of acute phase proteins [16]. It is believed [23] that TNF-α regulates the cytokine cascade and is the cause of inflammation and local destructive reactions. Proinflammatory mediators, in particular TNF-α, through the mechanisms of OS and activation of various molecular and/or metabolic pathways induce insulin resistance [16], promote cell degeneration, initiate inflammatory processes in the walls of arterial vessels and their calcification [9]. It has been shown that the multipotent cytokine TNF-α in patients with type 2 DM, affecting the endothelium, enhances the expression of cell adhesion molecules [24], significantly reduces the formation of basal NO [25] and induces apoptosis and differentiation of endothelial cells [24]. It should be noted that in patients with DN, endothelial damage is the earliest sign, which precedes the change in its integrity and the appearance of micro- and macrovascular complications, reaching its apogee in case of stage 5 [26]. It is known that ED is an integrated syndrome of insulin resistance and is observed in 80–90% of patients with CKD, deepens it, increases vascular reactivity, provoking endothelial disorders [27].

The systemic character of endothelial damage in patients with DN treated with HD has been evidenced by an increase in the number of CECs, which are an index of the final stage of endothelial cell activity and reflect the degree of their destruction. Maybe, under conditions of accelerated cell apoptosis, patients with type 2 DM with kidney damage disrupt the regeneration and utilization of desquamated endothelium, which may be one of the reasons for the decrease in one of the stable metabolites NO – NO_2- [28]. Important in the progression of ED with the formation of NO deficiency in HD patients with DN, in addition to inflammatory mechanisms [24, 29], is the accumulation of endogenous inhibitor of endothelial NO-synthase (eNOS) of asymmetric dimethylarginine (ADMA), accumulation of atherogenic lipoproteins, as well as the development of OS [4]. Importantly, uremic toxins, in particular sulfate-p-cresyl and ADMA, in patients with CKD VD stage are significantly associated with DN and

cardiovascular disease [4, 5], can adversely affect the reduction and dysfunction of endothelial progenitor cells, induce aging of endothelial cells, promote neoangiogenesis and vascular calcification [30]. Probably, the high frequency of vascular endothelial abnormal reactions and low mean EDVD BA values in case of DN and programmed HD are explained by depletion and distortion of compensatory "dilating" reactivity of endothelium to normal stimuli, imbalance between synthesis of vasodilators, in particular NO, and vasoconstrictors in favour of the latter or a complete loss of regulation of the diameter of artery relative to the shear stress [31].

Under the conditions of activation of free radical processes, severe dyslipidemia and ED [4, 23] enhanced synthesis of CRP and FG (caused by TNF-α, IL-6 and other cytokines) in HD patients with DN, apparently accelerates the mechanisms of atherogenesis, MIA-syndrome and thrombotic events [8,9]. The pathogenetic significance of CRP, like most cytokines, is realized through the induction of the nuclear factor kappa-B (NF-κβ) system [16], which is involved in the activation of specific proinflammatory genes and cell apoptosis mechanisms and is the subject of increased attention of scientists in terms of full understanding of ways of formation of cardiovascular pathology in case of DN. Moreover, CRP reduces the production/expression of eNOS and can directly lead to ED [31,32]. Interestingly, magnesium deficiency is associated with NF-κβ activation, decreased NO bioavailability, cytokine aggression, and progression of atherosclerosclerotic damage [33,34,35]. Hyperfibrinogenemia in case of CKD may be associated with inflammation, fibrin formation, increased blood viscosity, increased platelet aggregation, thrombophilia, and stimulation of smooth muscle cell proliferation [8, 9]. It is also important that HD patients with high levels of FG and atherosclerotic damage to the carotid arteries have a high risk of developing acute cerebral insufficiency [36].

Thus, activation of endothelial cell apoptosis, decreased generation (NO_2-) of NO and significant correlation of inflammatory markers with indicators of structural and functional state of endothelium (especially with CECs) in our study today suggest that the endothelium is a key modulator of inflammation in patients with type 2 DM with kidney damage on programmed HD. The increase in the ratio of angiopoietin 2 to angiopoietin 1, the growth of $CD14^{+}/CD16^{++}$ and $CD14^{++}/CD16^{+}$ subpopulations of monocytes, as well as the accumulation of $CD31^{+}$ Annexin V^{+} microvesicles in DN and HD confirms this thesis and indicates the predictive value of endothelial damage [37]. The pathogenetic association of proinflammatory markers with left ventricular hypertrophy, systolic and diastolic dysfunction, pulmonary hypertension, central and peripheral artery damage in patients with CKD VD stage has been demonstrated in many [38, 39], but not all [40] studies. There is microvascular inflammation of endothelial cells, reduced bioavailability of NO, including in cardiomyocytes [10]. It is possible

that in conditions of hyperglycaemia and insulin resistance the complex process of remodeling of cardiovascular system in HD is regulated by numerous genes that are responsible for functional activity of endothelium, accumulation of atherogenic lipoproteins, development of inflammatory reactions, rearrangement of extracellular matrix, vasculogenesis and ectopic calcification. Increased desquamated endothelial cell counts, activation of TNF-α and enhanced CRP and FG production are apparently factors in an unfavorable prognosis (due to the formation of a very high cardiovascular risk) in DN patients treated with HD, which deepens our understanding [6] and confirms the findings of other studies. [23, 32, 41].

Conclusions

1. DN in patients with programmed HD is combined with the activation of chronic inflammation, which is manifested by an increase in the content of proinflammatory cytokine TNF-α, an increase in the concentration of acute phase proteins CRP and FG and a simultaneous slight decrease in albumin levels.

2. Typical manifestations of ED in patients with type 2 DM with kidney damage in the case of HD are endothelial damage (CECs accumulation), disturbance of vasoreactivity with frequent development of pathological reactions of the vascular endothelium (low EDVD BA) and NO deficiency (decreased NO_2-).

3. Indices of activity of chronic inflammation and structural and functional state of the endothelium in case of DN in patients with CKD VD stage are closely related.

4. Proinflammatory mediators, in particular TNF-α, CRP, and the amount of CECs in patients with type 2 DM with kidney damage in HD can be considered as predictors of progression of cardiovascular complications, as well as prognostic markers, including the evaluation of therapeutic strategies and timely correction of inflammation and ED.

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Z.I. Litovkina: selection of patients for study, analysis of the received data, registration of the text of work and preparation of article for the press.

O.B. Susla: concept and design of the study, formulation of conclusions, approval of the final version of the article.

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