

AUTOIMMUNE MECHANISMS OF CHRONIC HEART FAILURE PROGRESSION IN ARTERIAL HYPERTENSION AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE WITH CONCOMITANT TYPE 2 DIABETES MELLITUS*

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Cardiovascular diseases are the main cause of the death in Europe, the USA and Asia [1,2]. Essential hypertension (EHT) and chronic obstructive pulmonary disease (COPD) are rather common cardiovascular diseases leading to CHF.

Today the attention of the scientists in many countries is drawn to the role of autoimmune mechanisms in the development and progression of many diseases. Of great importance is to determine the role of specific antibodies against the structural components of the host tissues, since they are able to make a direct damaging effect on the cells, resulting in the loss of their functional activity.

It is well known, that extracellular matrix proteins play an important role in the remodeling of the airways and blood vessels, and type IV collagen as a main component of basal membranes of endothelial cells, is important in the communications between the components of extracellular matrix and bloodstream.

Deterioration of polymer molecular structure of type IV collagen is well known in Goodpasture's syndrome. However, a number of researches showed that type IV collagen may accumulate in other diseases. Thus, in

type 1 diabetes mellitus its accumulation in the vascular wall leads to disruption of the vascular wall barrier and stimulates thrombosis [3]. This excess of type IV collagen in subendothelial space leads at first to the extravasation, and then — to the induction of protein paravascular fibrosis. Thus, accumulation of type IV collagen is regarded as one of the causes of microangiopathy in type 1 diabetes mellitus, including nephropathy. Moreover, Nikolov A. et al. showed the association of increased levels of autoantibodies against type IV collagen with microangiopathies and the development of diabetes mellitus [4].

Recent studies have shown that most patients with COPD have elevated levels of serum antibodies that react with autoantigens [5,6], and the appearance of antibodies to specific autoantigens correlates with the severity of the disease [7].

The studies of an autoimmune etiology of COPD give inconsistent results. Thus, Toyoshima M. et al. suggested that autoantibodies in COPD occur due to a general inflammation and did not play a significant role in the pathology of the disease [8]. However, Feghali-Bostwick CA et al. detected the autoantibodies

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to lung epithelium in the serum of patients with COPD [5]. Kirkham P. A. et al. reported that carbonyl-modified proteins resulting from oxidative stress, contribute to the formulation of autoantibodies, reflecting the ability to manage oxidative stress and autoimmune response in COPD [7]. An autoimmune origin of the lung remodeling was clearly demonstrated by Lee S. H. and colleagues, who determined that the severity of emphysema in COPD was significantly associated with the level of antibodies to elastin [9]. Later the association of emphysema in COPD patients with high levels of autoantibodies to aggrecan and collagen molecules was discovered and Rinaldi M. et al. indicated, that the autoimmune response to collagen and not elastin was associated with the severity of COPD [10].

Immunohistochemical studies in patients with COPD have demonstrated an increase in the accumulation of type IV collagen in neointima [11].

According to Packard T.A. in patients with COPD the level of antibodies to type IV collagen is 2 times more of the value of the healthy individuals. Daffa N.I. et al. also reported of the increased antibodies to type IV collagen in COPD [12]. But the relationships of those data with clinical and functional parameters of the heart and lungs the authors have not performed.

Therefore it has been accumulated enough data to suggest that the autoimmune response is present in patients with COPD and has an association with the disease progression [13].

Autoantibodies against type IV collagen have also been identified in cardiological pathology. According to the Tandon R., carditis in rheumatic fever is the result of the im-

mune response against type IV collagen by causing its effects on endothelium [14]. The ability of streptococcal protein, responsible for the rheumatism, to bind type IV collagen was further demonstrated and the continuation of these findings was the discover of increased antibodies against type IV collagen in patients with acute rheumatic fever [15].

According to the recent reports patients with myocardial infarction have significantly elevated levels of IgM and IgG against native type IV collagen. The study of Matache C. et al. using ELISA method demonstrated the association of increased levels of IgG antibodies to type IV collagen with myocardial infarction and idiopathic dilated cardiomyopathy [16]. Nicoloff G. et al. found autoantibodies against type IV collagen in EHT combined with type 1 diabetes mellitus [17].

Deterioration of collagen metabolism plays an important role in the formation and progression of heart failure. Uncontrolled synthesis and accumulation of collagen in the interstitium of the myocardium and blood vessels leads to the increase of their hardness and remodeling. Despite the association of antibodies to type IV collagen with hyperglycemia, bronchial and cardiovascular risk and their role remains unclear in CHF on the background of cardio-pulmonary pathology, as well as their association with hemodynamic, morphological and functional parameters of the cardiovascular system. Therefore, the aim of the research was to examine whether autoantibodies against native or aldehyde-modified type IV collagen are associated with heart failure progression in arterial hypertension combined with chronic obstructive pulmonary disease and type 2 diabetes mellitus.

MATERIALS AND METHODS

A total of 91 patients with CHF were recruited in the study. Group 1 consisted of 32 patients with CHF on the background of COPD and EHT, Group 2 comprised 32 patients with CHF on the background of COPD, EHT and type 2 diabetes mellitus. The average age of the first group was 63.5 ± 3.4 years, of the second group — 60.2 ± 2.6 years. Both groups were comparable by duration of

CHF (mean 5.3 ± 1.4 years) and EHT (mean 8.4 ± 2.1 years). 65.6% of patients developed type 2 diabetes mellitus after existing heart failure, and in 34.4% of patients CHF occurred on the background of diabetes. The groups were further subdivided into 2 groups according to the II and III functional class (FC) of CHF by NYHA. 27 patients with CHF on the background of EHT stage II served

as a reference group (Group 3) with mean age 60.74 ± 1.62 years. Group 1, 2 and 3 were adjusted by male/female ratio. 20 healthy volunteers adjusted for age and sex served as controls.

The exclusion criteria were: oncological diseases, tuberculosis, acute respiratory tract infections, strokes and myocardial infarctions in anamnesis. The protocol of the research was approved by the local ethical committee of the University.

The diagnosis of COPD of Groups B and C was established on the basis of typical symptoms, modified Medical Research Council (mMRC) dyspnea scale, COPD Assessment Tool (CAT) and spirometry according to GOLD 2013 guidelines [18]. The values of Forced expiratory volume in first second (FEV1) less than 80% of the expected value and ratio of forced expiratory volume in first second to the forced vital capacity (FEV1/FVC) less than 0.7 (70%) after post bronchodilator inhalation were included in this study. According to the degree of airflow limitation COPD 2 and COPD 3 degrees of bronchoobstruction were distinguished. Diagnosis of type 2 diabetes mellitus was carried out according to the criteria of International Diabetes Federation, recommendations of American Diabetes Association and European Association for the Study of Diabetes. The patients suffering from type 2 diabetes mellitus had mild to moderate severity of the disease in a state of subcompensation (fasting glucose level < 7.6 mmol/l, postprandial glucose level < 9.0 mmol/l, $HbA_{1c} < 8,5\%$). The duration of type 2 diabetes mellitus varied from 1 to 20 years.

Only COPD patients with EHT of II stage were enrolled in the study. Diagnosis of EHT was established according to ESH/ESC crite-

ria that consider as hypertension blood pressure values over 140/90 mmHg.

All the patients underwent echocardiography. Ultrasound investigation of the heart included detection of left atrium diameter (LA), left ventricle end systolic diameter, left ventricle end diastolic diameter, left ventricle end systolic volume, left ventricle end diastolic volume, left ventricle ejection fraction, right atrium and right ventricle diameter, pulmonary artery pressure.

A sample of peripheral venous blood was taken from fasting patients. Participants were instructed to take their medications as usual in the morning of the examination. Venous blood was citrated, centrifuged and stored at -20°C until the laboratory investigation of plasma collagen type IV antibodies.

Plasma IV type collagen antibodies concentrations were measured with enzyme-linked immunosorbent assay (ELISA) method, using antibody-coated microwell plate kit for *in vitro* diagnostic («ИМТЕК», Russia), according to the manufacture instructions.

Standards, controls, and sample assays were performed in duplicate. The standard calibration curve was calculated using mean replicate values of six standard calibrators with different levels of concentration. The intrassay coefficient of variation (CV) was lower than 4.5%. The interassay CV varied from 4.3% for high concentrations to 7.4% for low concentrations. All values are expressed as mean and standard error of the mean ($M \pm m$). Statistical analysis was done using computer program Statistica for Windows 10.0. Pearson's coefficient was used in the correlation analysis. Mann-Whitney criterium and ANOVA were used to assess the differences between groups. The level of significance was at $p < 0.05$.

RESULTS AND DISCUSSION

Patients with CHF at EHT (Group 3) showed increase of the level of type IV collagen antibodies on 28.2% as compared to controls ($p < 0.05$). Patients with CHF at EHT and COPD (Group 1) demonstrated a significant increase of type IV collagen antibodies — on 82% in comparison to healthy controls

($p < 0.05$) and on 42.0% when compared with patients at CHF on the background of EHT ($p < 0.05$), indicating that autoimmune reactions are more expressed in CHF in the presence of COPD (Table 1).

Group 2 — patients with CHF at EHT, COPD and type 2 diabetes mellitus showed

the highest level of type IV collagen antibodies — on 20.2 % when compared to the patients with CHF at EHT and COPD without type 2 diabetes mellitus ($p < 0.05$), that testifies of more expressed autoimmune processes induced by type 2 diabetes mellitus.

While CHF progression from the II class to the III class by NYHA the patients with EHT and COPD revealed increase of type IV collagen antibodies on 26.5 %, while patients with EHT, COPD and type 2 diabetes mellitus — on 29.6 ($p < 0.05$) (Table 2).

It is known, that EHT and diabetic vascular complications are associated with an elevated degradation of connective tissue. As a result type IV collagen is released into the circulated blood, which is a pathological stimulus for an increased production of type IV collagen antibodies.

Thus, hyperglycemia causes not only direct damaging effect on the components of connective tissue, but also initiates an autoimmune response to its components, which is associated with the progression of CHF.

Correlation analysis with cardiohaemodynamic parameters in patients with CHF

at EHT, COPD and type 2 diabetes mellitus revealed significant association between type IV collagen antibodies and end systolic diameter of the left ventricle ($r = 0.44$), end diastolic diameter of the left ventricle ($r = 0.37$), end systolic volume of the left ventricle ($r = 0.45$), end diastolic volume of the left ventricle ($r = 0.35$), left atrium diameter ($r = 0.30$) and ejection fraction of the left ventricle ($r = -0.44$) ($p < 0.05$). Analysis of the parameters of the right heart showed direct correlation between type IV collagen antibodies and sizes of the right atrium ($r = 0.53$), right ventricle ($r = 0.52$) and pulmonary artery pressure ($r = 0.51$) ($p < 0.05$).

Thus, the processes of the left and the right heart dilation, presence of pulmonary hypertension are associated with high levels of antibodies to type IV collagen, that may indicate their damaging effect on the metabolism of connective tissue in the myocardium, since collagen IV is widely expressed in the heart tissue. This is consistent with the data of Matache C. et al. demonstrated the association of raised antibodies against type IV collagen with idiopathic dilated cardiomyopathy [17].

Table 1

The level of type IV collagen antibodies in patients with chronic heart failure in essential hypertension and essential hypertension combined with chronic obstructive pulmonary disease with and without type 2 diabetes mellitus ($M \pm m$)

Parameter	Group 1 (n = 32)	Group 2 (n = 32)	Group 3 (n = 27)	Group 4 (Controls) (n = 20)	<i>p</i>
Type IV collagen antibodies (μ /ml)	10.07 \pm 0.32	12.11 \pm 0.4	7.09 \pm 0.36	5.53 \pm 0.2	$p_{1-2} < 0.05$ $p_{1-3} < 0.05$ $p_{1-4} < 0.05$ $p_{2-3} < 0.05$ $p_{2-4} < 0.05$ $p_{3-4} < 0.05$

Table 2

The level of anti-collagen type IV antibodies in patients with essential hypertension and chronic obstructive pulmonary disease with and without type 2 diabetes mellitus while chronic heart failure progression ($M \pm m$)

Parameter	CHF class II by NYHA	CHF class III by NYHA	<i>p</i>
EHT and COPD with type 2 diabetes mellitus	10.62 \pm 0.43 (n = 17)	13.77 \pm 0.51 (n = 15)	$p_{1-2} < 0.05$
EHT and COPD without type 2 diabetes mellitus	9.28 \pm 0.36 (n = 16)	11.74 \pm 0.48 (n = 16)	$p_{1-2} < 0.05$

CONCLUSIONS

1. Correlation of the levels of type IV collagen antibodies with cardiac morphofunctional parameters indicate of their pathogenetic importance in the development of myocardial dysfunction and may be used in the diagnosis, control of the course and prognosis of chronic heart failure in essential hypertension and chronic obstructive pulmonary disease with and without type 2 diabetes mellitus.
2. The study of the mechanisms of the direct damaging effect of type IV collagen antibodies in chronic heart failure and the development of therapeutic strategies to control their level is an interesting direction for further studies.

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АУТОІМУННІ МЕХАНІЗМИ ПРОГРЕСУВАННЯ ХРОНІЧНОЇ СЕРЦЕВОЇ НЕДОСТАТНОСТІ ПРИ АРТЕРІАЛЬНІЙ ГІПЕРТЕНЗІЇ ТА ХРОНІЧНОМУ ОБСТРУКТИВНОМУ ЗАХВОРЮВАННІ ЛЕГЕНЬ ІЗ СУПУТНИМ ЦУКРОВИМ ДІАБЕТОМ 2 ТИПУ

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Проведено дослідження взаємозв'язку аутоантитіл до колагену IV типу з прогресуванням хронічної серцевої недостатності при артеріальній гіпертензії та хронічному обструктивному захворюванні легень в залежності від наявності цукрового діабету 2 типу. Виявлено, що у таких хворих має місце збільшення рівня аутоантитіл до колагену IV типу в порівнянні зі здоровими особами. Рівень аутоантитіл до колагену IV типу показав достовірну кореляцію зі збільшенням розмірів і об'ємів міокарду лівого шлуночка та зниженням його скоротливої здатності. Підвищення рівня аутоантитіл до колагену IV типу асоційовано з прогресуванням серцевої недостатності з артеріальною гіпертензією, хронічним обструктивним захворюванням легень, в сполученні з цукровим діабетом 2 типу.

Ключові слова: аутоантитіла проти колагену IV типу, цукровий діабет 2 типу, хронічна серцева недостатність, хронічне обструктивне захворювання легень, артеріальна гіпертензія.

**АУТОИММУННЫЕ МЕХАНИЗМЫ ПРОГРЕССИРОВАНИЯ ХРОНИЧЕСКОЙ
СЕРДЕЧНОЙ НЕДОСТАТОЧНОСТИ ПРИ АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИИ
И ХРОНИЧЕСКОМ ОБСТРУКТИВНОМ ЗАБОЛЕВАНИИ ЛЕГКИХ
С СОПУТСТВУЮЩИМ САХАРНЫМ ДИАБЕТОМ 2 ТИПА**

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Проведено исследование взаимосвязи аутоантител к коллагену IV типа с прогрессированием хронической сердечной недостаточности при артериальной гипертензии с хроническим обструктивным заболеванием легких в зависимости от наличия сахарного диабета 2 типа. Выявлено, что у этих больных имеют место высокие уровни аутоантител к коллагену IV типа в плазме крови по сравнению со здоровыми лицами. Уровень аутоантител к коллагену IV типа показал достоверную корреляцию с увеличением размеров и объемов миокарда левого желудочка и снижением его сократительной способности. Повышение уровня аутоантител к коллагену IV типа ассоциировано с прогрессированием хронической сердечной недостаточности с артериальной гипертензией, хроническим обструктивным заболеванием легких, в сочетании с сахарным диабетом 2 типа.

К л ю ч е в ы е с л о в а: аутоантитела к коллагену IV типа, сахарный диабет 2 типа, хроническая сердечная недостаточность, хроническое обструктивное заболевание легких, артериальная гипертензия.

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We examined whether autoantibodies against type IV collagen are associated with heart failure progression in arterial hypertension combined with chronic obstructive pulmonary disease with and without type 2 diabetes mellitus. Both patients with chronic heart failure in essential hypertension and chronic obstructive pulmonary disease and patients with concomitant type 2 diabetes mellitus showed higher levels of plasma type IV collagen autoantibodies in comparison to healthy controls. Type IV collagen antibodies correlated with dilation of the left ventricle and reduction of its contractility. Elevation of plasma type IV collagen autoantibodies is associated with progression of chronic heart failure in arterial hypertension combined and chronic obstructive pulmonary with and without type 2 diabetes mellitus.

Key words: autoantibodies against type IV collagen, type 2 diabetes mellitus, chronic heart failure, chronic obstructive pulmonary disease, arterial hypertension.