УДК 616-004.8

DYNAMICS OF RENAL FIBROSIS MARKERS IN PATIENTS WITH CHRONIC HEART FAILURE

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Aims *Determination of collagen IV in urine sediment and pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) in the blood for the purpose of early detection of sclerotic processes in the glomerular membrane of the kidneys in patients with chronic heart failure (CHF).*

Materials and methods *The experiment included 225 patients with functional classes (FC) II-III of CHF of ischemic origin with reduced and mid-range ejection fraction (EF) according to the New York Heart Association (NYHA) classification. The average age was 64.3+ 0.62, 135 (60%) patients were men, 90 (40%) women. Patients were divided into 3 groups according to their treatment tactics. Group I - 72 patients in complex treatment received additional sacubitril + valsartan 50 mg / day, group II - 77 patients in complex treatment with empagliflozin 10 mg / day, group III - 76 patients - a combination of sacubitril + valsartan and empagliflozin.*

Results *Microalbuminuria was detected in 36.4% of the patients we examined, normoalbuminuria in 53.1%, and macroalbuminuria in 10.1% of patients. Our examination showed that all 100% of patients had collagen IV excretion to varying degrees. When determining the urinary excretion of type IV collagen, some correlations were also identified. Thus, its content in urine is highly negatively correlated with glomerular filtration rate (GFR) ($r = -0.742$; $P<0.001$) and significantly positively correlates with albuminuria ($r = -0.683$; $P<0.001$). In the group of patients receiving sacubitril-valsartan in combination, TNF-α levels significantly decreased over 3 months from 13.22 to 7.8 pg/ml; in the group of patients receiving empagliflozin in combination, TNF-α levels significantly decreased from 12.6 to 7.2 pg/ml, in the group where both sacubitril-valsartan and empagliflozin were used, the TNF-α indicator significantly decreased from 12.8 to 5.6 pg/ml. Evetually, pathogenetic therapy of CHF leads to stabilization of proinflammatory cytokines.*

Conclusion *The detection of collagen IV in patients with CHF with class II-III in the urine suggests the important role of this marker as an independent predictive factor for the development of glomerulosclerosis. The use of*

a combination of drugs empagliflozin and sacubitril-valsartan in complex treatment has a beneficial effect on renal filtration by stabilizing pathological processes which can lead to slow down the processes of fibrosis formation and glomerulosclerosis in the basement membrane and renal tubules. TNF-α indicators were significantly reduced with a greater effect in the third group of patients with CHF, where a combination of sacubitril-valsartan and empagliflozin was used. In this connection, we can assume that empagliflazin also has an anti-inflammatory effect and, in combination with sacubitril-valsartan, has a pronounced anti-inflammatory, and subsequently anti-fibrotic effect.

Keywords: *chronic heart failure, collagen IV, glomerulosclerosis, renal fibrosis, chronic kidney disease, proinflammatory cytokine, Tumor necrosis factor-alpha.*

It is well known that the first place among morbidity in the world is occupied by cardiovascular pathology, of which a significant part is heart failure. According to some European researchers, the prevalence of CHF in 2020 in Europe was 1.3 to 4.0% in the general population, of which in Germany 4.0%, in Sweden 2.2%, and in China 1.3-3.5%, in the USA 2.4-2.6% [2, 4]**.** According to numerous literature data, CHF leads to the formation of cardiorenal syndrome, which results in the development of chronic kidney disease (CKD).

To date, more than 800 million patients with CKD have been registered worldwide, and in Uzbekistan their number is 102,969 patients. More than 1.2 million people die from it every year around the world. Scientists predict that by 2040, mortality from CKD will increase by 150% compared to 2016 [3]**.**

Numerous studies indicate that as a result of venous stagnation that develops in CHF, central venous pressure increases and filtration pressure in the capillaries of the glomeruli of the kidneys decreases, which leads to a decrease in the glomerular filtration rate, as a consequence of hyperfiltration [12]. As a result of the above processes, a structural and functional restructuring occurs in the glomerulo-basilar membrane - spreading of the legs and processes and epithelial-mesenchymal transdifferentiation of podocytes, resulting in cell apoptosis [9, 5].

According to some scientists, it has been proven that podocytes are not capable of proliferation and replacement, therefore the progressive loss of these cells in the glomerulus of the kidney entails exposure of the glomerulobasilar membrane and triggers the processes of glomerulosclerosis [11, 9, 5]**.** As a result of detachment of podocytes from the glomerulo-basilar membrane, they are excreted into the urinary space, where their fragments, protein nephrin and collagen IV, are detected as markers of apoptosis and glomerulosclerosis [6, 10]**.**

Type IV collagen constitutes the main structure of the basement membrane and mesangial matrix of the glomerular kidney, is a high molecular weight fibrillar protein with a molecular weight of approximately 540 kDa, consists of α3, α4 and α5 chains and is generally not filtered through the glomerular basement membrane.

Therefore, the determination of collagen IV in urine sediment is great scientific and practical importance for the early detection of sclerotic processes in the glomerular membrane of the kidneys in patients with CHF.

In addition, one of the mechanisms for the development of fibrosis in the kidneys and glomerulosclerosis in patients with CHF is also the activation of pro-inflammatory cytokines, which lead to the progression of this pathology.

Proinflammatory cytokines have a major role in inflammatory diseases of infectious or non-infectious origin. Pro-inflammatory cytokines include IL-1, IL-6, IL-8, IL-12, IFN-γ, IL-18 and one of the most important - TNF-α. These cytokines localize and resolve inflammation by activating local and systemic inflammatory responses. TNF-α also triggers the cytokine cascade of anti-inflammatory cytokines. In most cases, the inflammatory reaction is successfully eliminated. Excessive production of cytokines or failure to stop the production of proinflammatory cytokines, however, can lead to an increase in the number of cytokines in the systemic circulation, which is called a “cytokine storm”; continued production of cytokines can have harmful effects on the human body with the development of hypotension, intravascular thrombosis, pulmonary edema and hemorrhages; if left unchecked, this process can lead to multiple organ failure and death, a condition often called systemic inflammatory response syndrome (SIRS). [12].

The detection in the blood of patients with heart failure, regardless of its etiology, of increased levels of pro-inflammatory cytokines, which have a number of negative effects, made it possible to formulate the immunoinflammatory concept of the pathogenesis of CHF. The body’s immune defense is “triggered” not only by various types of infectious or viral aggressions, but also responds to any stressful influence, such as myocardial ischemia, hemodynamic overload, intoxication, etc., that is, to those factors that led to the development of CHF . It has been established that TNF-α is able to stimulate left ventricular hypertrophy (LVH), increase the content of CRP, activate coagulation, worsen the lipid spectrum of the blood, and most importantly, activate fibroblasts and trigger collagen synthesis, which leads to fibrosis both in cardiomyocytes and glomerulosclerosis in the kidneys. Simultaneously with the pro-inflammatory effects, TNF-α has a pronounced pro-atherosclerotic and pro-thrombogenic effect due to the stimulation of fibroblast proliferation, the production of metalloproteinases, and the accumulation of the main components of the extracellular matrix.

Materials and methods. The experiment included 225 patients with FC II-III of CHF of ischemic origin with reduced and mid-range ejection fraction (EF) according to the New York Heart Association (NYHA) classification. The average age was 64.3+ 0.62, 135 (60%) patients were men, 90 (40%) women. The patients were treated in the cardiology department of the Multidisciplinary Clinic of the Tashkent Medical Academy. In complex therapy, patients received angiotensin-converting enzyme (ACE) inhibitors or angiotensin-2 receptor antagonists, mineralocorticoid receptor antagonists (eplerenone or spironolactone), beta-blockers, loop diuretics and cardiac glycosides according to indications.

Patients were divided into 3 groups according to their conducting tactics. Group I - 72 patients in complex treatment received additional sacubitril + valsartan 50 mg / day, group II - 77 patients in complex treatment with empagliflozin 10 mg / day, group III - 76 patients - a combination of sacubitril + valsartan and empagliflozin.

Collagen IV studies were carried out using the Collagen Type IV alfa1 reagent from “Elabscince” (America) in the clinical laboratory of the TMA clinic.

TNF-α concentration using the “alpha-TNF-ELISA-BEST” (Vector-Best CJSC, Novosibirsk).

Research results and discussion.

As is known, one of the first signs of damage to the glomerular membrane of the glomeruli is albuminuria. For this reason, we, in parallel with collagen IV, determined albuminuria in the examined patients and the following changes were identified (Fig. 1).

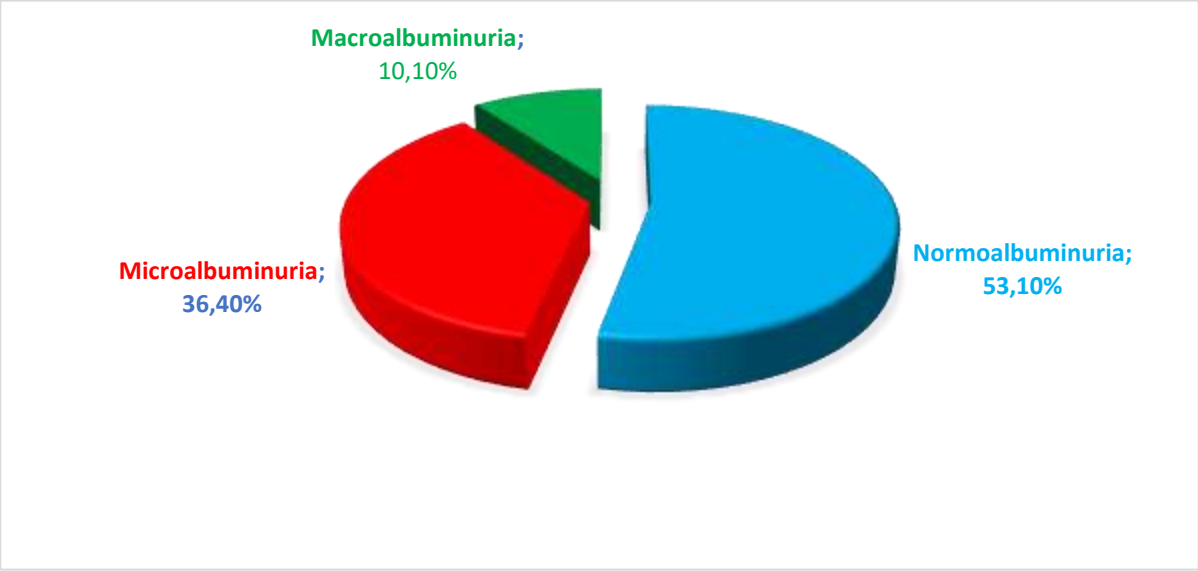


Fig.1. Albuminuria in patients with CHF with reduced and mid-range ejection fraction (%).

Microalbuminuria was detected in 36.4% of the patients we examined, normoalbuminuria in 53.1%, and macroalbuminuria in 10.1% of patients. As follows from the figure, we see that almost 2/3 of the patients had albuminuria. That is, it can be assumed that in almost all patients with FC II and III CHF there is a deterioration of the permeability of the glomerular basement membrane.

As part of this work, we conducted a comparative study of type IV collagen excretion in urine in patients with FC II and III CHF with reduced and mid-range EF. It turned out that in the patients studied, the detection of type IV collagen was observed along with patients who had microalbuminuria, and in patients with normal protein excretion. This reflects the initial stages of accumulation of this type of collagen in the kidneys. The relationship between an increase in urinary excretion of type IV collagen and an increase in the volume of glomerular mesangium in db/db mice (model of T2DM) was previously described [1]. In patients with T2DM, excretion of type IV collagen was associated with morphological changes in the glomeruli, tubules and interstitium of the kidneys and reflected the accumulation of collagen in these structures [7].

Our examination showed that all 100% of patients had collagen IV excretion to varying degrees. Interestingly, in those patients who had normoalbuminuria, the presence of collagen IV was also detected in the urine, which suggests the important role of basal excretion of type IV collagen in the urine as an independent predictive factor for the development of glomerulosclerosis with subsequent renal failure. Its presence in the urine of patients with CHF, even with normoalbuminuria, allows it to be considered an early non-invasive marker for diagnosing fibrosis processes in the kidneys.

The urinary excretion of collagen is more associated with the development of renal fibrosis, while albuminuria reflects the permeability of the renal filter. According to our data, collagen excretion is observed in all patients with FC II and III CHF with reduced and mid-range EF. These patients are likely to be at risk for further development of renal failure.

When determining the urinary excretion of type IV collagen, some correlations were also identified. Thus, its content in urine is highly negatively correlated with GFR (r = -0.742; P<0.001) and significantly positively correlates with albuminuria (r = -0.683; P<0.001; Fig. 2,3).

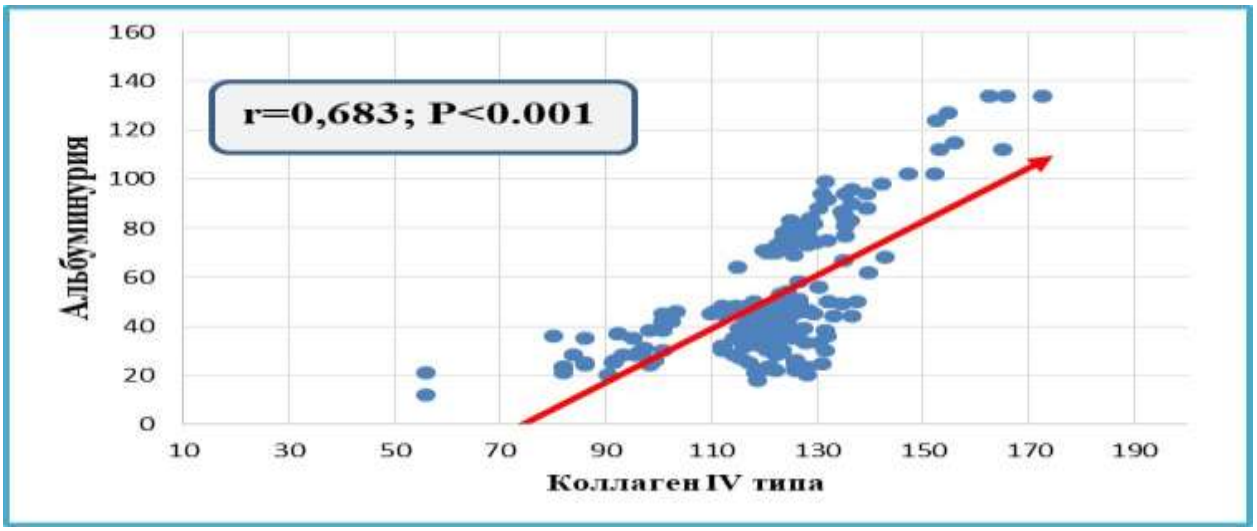


Fig. 2 Correlation between collagenuria and albuminuria in patients with chronic heart failure of functional classes II-III with reduced ejection fraction

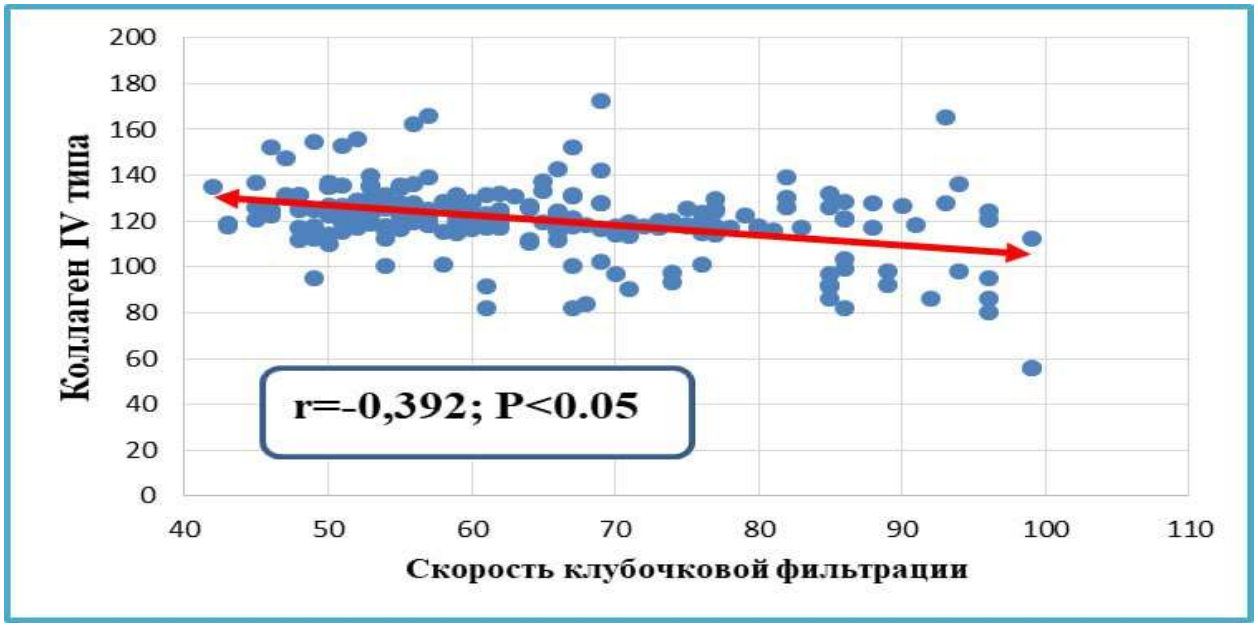


Fig. 3 Correlation relationships between collagenuria and glomerular filtration rate in patients with chronic heart failure of functional classes II-III with reduced ejection fraction

Further in our work, we examined the effect of various treatment tactics on the dynamics of collagenuria. Thus, before the start of treatment, the collagen content in urine averaged 91.8 ± 2.9 μg/mmol. After 10 days of complex treatment with the inclusion of sacubitril-valsartan, the collagen content in the urine decreased to 86.7±2.4 μg/mmol (p≥0.05). In the second group of patients who received empagliflozin as part

of complex treatment, it decreased from 93.2 ± 3.2 to 82.6 ± 3.4 ($p<0.05$), and in the main group that received sacubitril-valsartan and empagliflazin, this indicator significantly decreased from 90.7±2.8 to 78.6±3.0. After 1 and 3 months of treatment in the first group, the content of collagen IV continued to decrease, amounting to 83.2 ± 2.8 ($p<0.05$) and 78.6 ± 3.1 ($p<0.01$), respectively. In the second group of patients who received empagliflozin as part of complex therapy, the content of collagen IV was 74.5±2.9 ($p<0.01$) and 71.8±3.4 ($p<0.01$) after 1 and 3 months, respectively. And in the main group, where patients took empagliflozin + sacubitril valsartan in complex therapy, in the dynamics of therapy, the content of collagen IV was 69.7 ± 3.2 $p<0.001$ and 62.4 ± 2.6 $p<0.001$, respectively (Table 1).

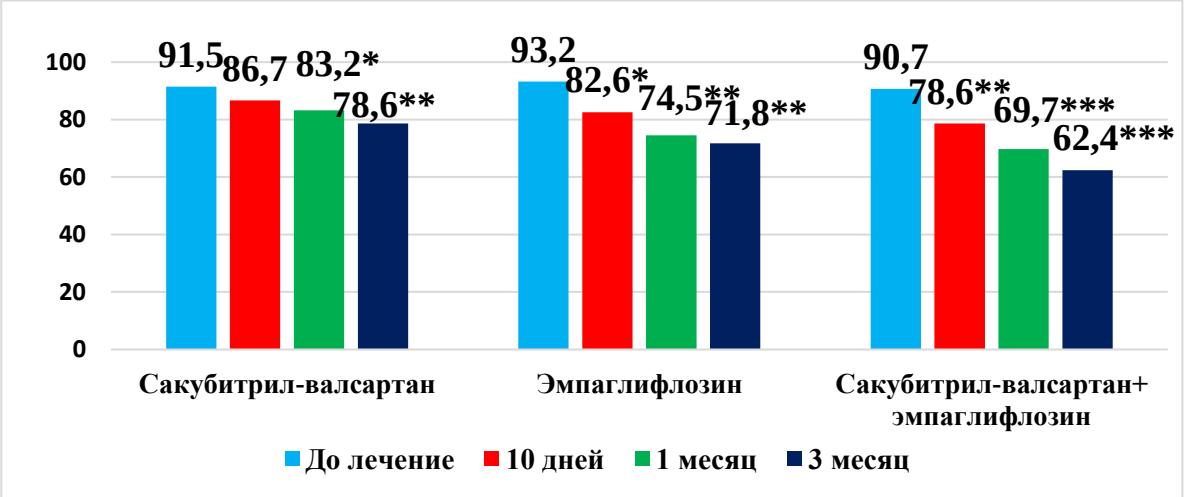
Table 1.

Level of collagen IV in urine in patients with chronic heart failure in the dynamics of therapy.

Indicators, collagen IV, mcg/mmol	**1-group, sacubitril-volsartan, n= 72**	**Group 2, empagliflozin n= 77**	**Group 3, empagliflozin + sacubitril-valsartan, n= 76**
Before treatment	91,5±2,6	93,2±3,2	90,7±2,8
10 days	86.7±2,4	82.6±3,4*	78.6±3,0**
1 month	83.2±2,8*	74.5±2,9**	69.7±3,2***
3 months	78.6±3,1**	71.8±3,4**	62.4±2,6***

Notes: reliability indicators before and after treatment *-p<0.05; ** p<0.01; *-p<0.001**

As can be seen from the graph (Fig. 4), in the main group of patients receiving empagliflozin + sacubitril valsartan, there was significant improvement in the content of collagen IV in the urine. The combination of these drugs has a beneficial effect on



renal filtration, by stabilizing the basal glomerular membrane which can slow down the processes of fibrosis formation in it.

Fig.4. Dynamics of changes in collagenuria in patients with chronic heart failure FC II and III with reduced and moderately reduced EF during therapy.

According to Panchapakesan U, Pegg K, it was shown that empagliflozin inhibits the synthesis of profibrogenic and inflammatory factors that are of key importance in the pathogenesis of renal dysfunction. When exposed to empagliflozin on immortalized human kidney cells HK-2, incubated under conditions of glucotoxicity in vitro, a decrease in the expression of type IV collagen was observed, which affects the expression of a number of pro-inflammatory and profibrotic factors) [8].

As can be seen from the above, an increase in TNF-α ultimately leads to fibrosis of cardiomyocytes as well as glomerulosclerosis in the renal glomeruli. To decide what the state of the cytokine-inflammatory state is in patients with CHF 2-3, the content of the pro-inflammatory cytokine TNF-α in the blood was studied in patients.

Table.2.

TNF-α indicators in patients with chronic heart failure before and after treatment.

TNF-α indicators, pg/ml	**Group 1, sacubitril-volsartan, n= 72**	**Group 2, empagliflozin n= 77**	**Group 3, empagliflozin + sacubitril valsartan, n= 76**
Before treatment	13,2±0,6	12,6±0,5	12.8±0.5
After 3 months	7,8±0,4**	7,2±0,3**	5,6±0,4***

As can be seen from Table 2, the average content of TNF-α in the three groups we studied was 13.2±0.6; 12.6±0.5; 12.8±0.5 pg/ml, this is almost 2 times higher than normal, which confirms the immunoinflammatory concept of the pathogenesis of CHF. In addition, it can be assumed that the increase in this indicator was also facilitated by the recently suffered systemic Covid-19-mediated inflammation, which also contributed to the inflammation process.

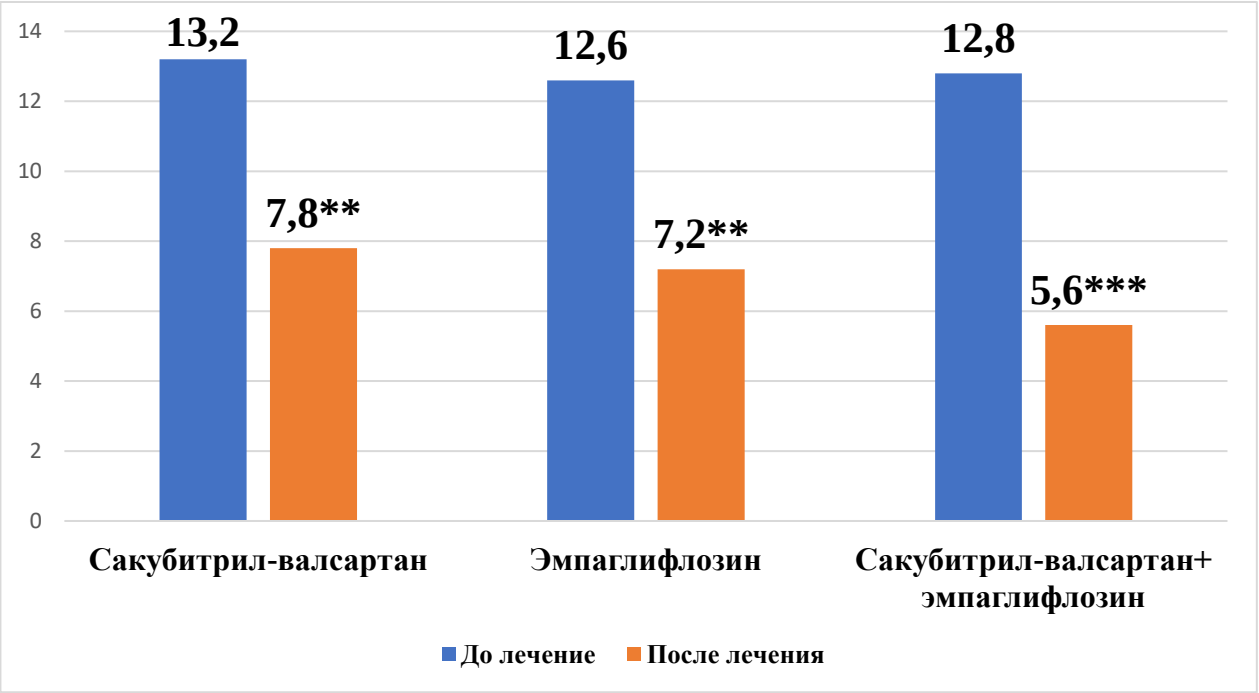


Fig.5. Dynamics of TNF-α indicators by group during treatment.

As can be seen from Fig. 5. significant positive changes are noted in the dynamics of the therapy. As mentioned above, the study patients received standard complex therapy consisting of ACE inhibitors or ARBs, beta blockers, mineralocorticoid receptor antagonists, and loop diuretics as needed. In the group of patients receiving sacubitril-valsartan in combination, TNF-α levels significantly decreased over 3 months from 13.22 to 7.8 pg/ml; in the group of patients receiving empagliflozin in combination, TNF-α levels significantly decreased from 12.6 to 7.2 pg/ml, in the group where both sacubitril-valsartan and empagliflozin were used, the TNF-α indicator significantly decreased from 12.8 to 5.6 pg/ml. Evetually, pathogenetic therapy of CHF leads to stabilization of proinflammatory cytokines.

Based on the above, the following conclusions can be drawn:

1. In the majority of patients with CHF II and III FC with reduced and mid-range EF, collagenuria is detected, which indicates that renal dysfunction and glomerulosclerosis processes have already begun, which begin long before the appearance of albuminuria in the examined patients.

2. The detection of collagen IV in patients whose urine had normoalbuminuria suggests an important role for the basal excretion of this marker as an independent predictive factor for the development of glomerulosclerosis with subsequent renal failure.

3. The role of collagen IV in patients with FC II and III CHF with reduced and mid-range EF as an early marker of renal fibrosis is confirmed by a highly negative correlation with GFR (r=-0.742; P<0.001) and a noticeably positive correlation with albuminuria (r=-0.683; P <0.001)

4. The content of TNF-α in the three groups we studied was 13.2±0.6; 12.6±0.5; 12.8±0.5 pg/ml, this is almost 2 times higher than normal, which confirms the immunoinflammatory concept of the pathogenesis of CHF.

5.The use of a combination of drugs empagliflozin and sacubitril-valsartan in complex treatment has a beneficial effect on renal filtration by stabilizing pathological processes which can lead to slow down the processes of fibrosis formation and glomerulosclerosis in the basement membrane and renal tubules.

6. As can be seen from the data of our study, TNF-α indicators were significantly reduced with a greater effect in the third group of patients with CHF, where a combination of sacubitril-valsartan and empagliflozin was used. In this connection, we can assume that empagliflazin also has an anti-inflammatory effect and, in combination with sacubitril-valsartan, has a pronounced anti-inflammatory, and subsequently anti-fibrotic effect.

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