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Endothelial Dysfunction as a Factor in the Pathogenesis of Chronic Heart Failure of Various Genesis

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chronic heart failure;
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endothelial dysfunction;
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Abstract.

The objective of the research was to study the pathogenetic role of the levels of endothelin-1 (ET-1), tumor necrosis factor-alpha (TNF-alpha) and C-reactive protein (CRP) in the development of endothelial dysfunction (ED) in patients with preserved and lost renal function.

Materials and methods. The study involved 86 patients: Group I included 42 patients with arterial hypertension and chronic heart failure (CHF) IIA FC III; Group II consisted of 44 patients with terminal chronic kidney disease (stage V CKD) with concomitant CHF IIA FC III who were on treatment by outpatient program hemodialysis (HD).

Results. In patients of Group II the levels of ET-1 and inflammatory markers were significantly higher than those in the comparison and control groups ($p<0.001$ and $p<0.001$). In patients of Group I there was a direct moderate correlation between TNF-alpha and ET-1 ($r=0.48$; $p<0.05$) and between CRP and ET-1 ($r=0.56$; $p<0.05$). In Group II, the relation between TNF-alpha and ET-1 was significantly stronger ($r=0.58$; $p<0.05$); between the levels of CRP and ET-1 it was weaker ($r=0.37$, $p<0.05$). Increased levels of ET-1, TNF-± and CRP affected the development of ED in both groups.

Conclusions. It was found that CRP levels of TNF-alpha, ET-1 in patients on HD were significantly different from the same data in patients with preserved renal function, which is clearly associated with more pronounced signs of inflammation and ED in a cohort of dialysis patients. The impact of TNF-± in the development of ED in both groups was proved. Despite the higher level of CRP in patients with stage V CKD being corrected by HD with CHF, this biomarker had less impact on the prognosis of ED than in the general population of patients with CHF.

Chronic heart failure (CHF) occupies the first place among all cardiovascular diseases by the rate of increase in incidence. Nowadays, the prevalence of this syndrome constitutes 1-9%, the incidence of CHF increases with age and reaches 10-28% after the age of 65 [2, 8]. The prevalence of CHF is much higher in a population of patients with terminal chronic kidney disease (CKD) who are on hemodialysis (HD). Thus, CHF is diagnosed in almost one third of patients on HD [10]. Endothelium of blood vessels intima is important in the pathogenesis of CHF development and progression. It performs barrier, secretory, homeostatic functions and plays an important role in the processes of inflammation and remodeling of the cardiovascular system. Endothelial cells are sensitive to various damaging factors such as homeostasis disorders, increased concentration of free radicals, cholesterol and inflammatory mediators under the influence of which endothelial dysfunction (ED) may develop being caused by an imbalance between substances providing vascular tone and homeostasis of endothelium processes [4, 5, 13]. Under physiological conditions, the secretion of vasoconstrictors (angiotensin II, endothelin-1, free radicals, thromboxane A2) and vasodilators (nitric oxide, hyperpolarizing relaxing factor, prostacyclin I2) is balanced. However, this balance is disrupted with a predominance of vasoconstrictor reactions accompanied by increased vascular tone and local spasm under the influence of various risk factors. Activation of inflammatory mediators C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF-alpha) are closely related to oxidative stress and ED. According to many researchers, long-term exposure to damaging factors leads to a gradual deterioration and distortion of vasodilation compensatory mechanisms. Thus, endothelium launches mechanisms of vasoconstriction and proliferation in response to a normal stimulus [1, 4, 5, 13]. Among the endothelium-dependent vasoconstrictors endothelin-1 (ET-1) draws the attention of various scientists [1, 3, 6, 7, 11]. The main properties of ET-1 are its ability to affect not only the vascular tone causing vasoconstriction stable, but also myocardial contractile function, the value of preload and afterload, platelet aggregation and to stimulate the development of myocardial hypertrophy. ED is a characteristic feature accompanying CHF. Many studies have found that increased levels of CRP and TNF-alpha in blood serum are an independent predictor of CHF progression [1,13,14]. However, the impact of these inflammatory mediators on ED severity and CHF development in patients requiring replacement therapy is poorly studied.

The objective of the research was to determine the pathogenetic role of ET-1, TNF-alpha and CRP in patients with stage V CKD with concomitant CHF requiring HD and in patients with essential arterial hypertension (AH) with CHF and to define the impact of end-stage kidney disease on indicators of endothelial dysfunction.

Materials and methods of the research

The study involved 86 patients: Group I included 42 patients with AH and CHF IIA FC III (NYHA) who underwent hospital treatment in the therapeutic department of the University Clinic of IFNMU; Group II consisted of 44 patients with chronic kidney disease (stage V CKD) with concomitant CHF IIA FC III (NYHA) who underwent treatment by outpatient program hemodialysis (HD) in the Department of Extracorporeal Detoxification Methods and Chroniodyalisis at the Ivano-Frankivsk Regional Clinical Hospital and in Nephrology and Dialysis Center at the Kalush Central District Hospital. The average age of the patients constituted 50.8 ± 5.85, median of HD treatment duration was (3.58 ± 1.32) years. The examined patients included 49 (57%) men and 37 (43%) women. Control group consisted of 20 apparently healthy individuals.

HD was performed to patients according to the standard program (3 times a week for 4-5 hours) on Innova devices of “Gambro” company (Sweden) using a semi-synthetic dialyzers and bicarbonate buffer. Provided dialysis dose (eKt/V ratio) constituted at least 1.3.

Patients with severe heart rhythm disorders, poorly controlled diabetes were excluded from the research as well as patients who were on outpatient HD no more than one year, eKt / V ratio below 1.3, hemoglobin 90 GM/DL.

In addition to full blood count and biochemical blood assay, diurnal variations of arterial blood pressure (ABP) were determined in all patients by means of 24-hour blood pressure monitoring. The clinical condition of the patient was assessed by total points according to the rating scale of clinical state (Yu.V. Marieiev, 2000) and 6-minute walking test was conducted in order to clarify the functional class of CHF (according to NYHA criteria). CRP level was determined with the use of panel according to semiquantitative method of latex particle agglutination "CRP Latex Agglutination Test" (Granum, scientific and production laboratory Ltd, Ukraine). TNF-alpha was determined on reader PR2100 (Sanofi diagnostics Pasteur, France) using a set of "alpha-TNF-EIA-Best" (private limited company "Vector-Best", Russia). For the study of endothelial dysfunction, the level of ET-1 in serum was determined by enzyme-linked immunosorbent assay using "Biomedica" kit (Austria) on Stat-Fax 303 + analyzer. All patients participating in the study signed informed consent.

Statistical processing of the data was performed using "Statistica 8.0 for Windows" taking into account the test for normal distribution with the use of the Kolmogorov-Smirnov test. In case of normal distribution, mean values (M) and mean error of arithmetical average (m) as well as standard deviation (SD) were estimated. Student's t-test was used to compare the average values of two independent samples. Median and interquartile range (25% and 75%) were used in case of non-compliance of the normal distribution law for description of the features. Nonparametric Mann–Whitney U test was applied for comparative analysis. Correlation relationship was investigated according to Pearson method (normal distribution) and Spearman (in case of the absence of normal distribution). The difference was considered statistically significant at $p<0.05$.

Results of the research and their discussion

Analysis of clinical performance and laboratory characteristics (Table 1) found that anemia symptoms were detected in both research groups, however, they were more significant in the group of dialysis patients. Thus, indices of hemoglobin and the number of red blood cells were 1.13 and 1.21 times below normal in Group I of patients. Deviations of the studied indices were 1.32 and 1.37 times ($p<0.05$) below normal in Group II.

Table 1

Clinical performance and laboratory characteristics of the patients

Indices	Control n=20	Group I (AH with CHF) n=42	Group II (V stage CKD, on HD with CHF) n=44
Sex (M/W)	11/9	23/19	26/18
Age, years	37.8±4.37	54.6±5.47	49.1±6.12
AH duration, years	-	7.8±2.42	6.7±0.87
HD treatment, years	-	-	3.58±1.32
Hemoglobin, GM/DL	127.3±5.13	112.4±4.16*	96.4±3.07**^^
Erythrocytes, 10^{12}/l	4.68±0.34	3.89±0.09	3.41±0.64*
Leukocytes, 10^9/l	5.1±0.47	7.2±0.13	7.6±0.15
Erythrocyte sedimentation rate, mm/h	6.88±2.13	7.23±1.43	14.4±1.25**^^

Total protein, g/l	73.1±3.84	63.3±3.56*	58.4±2.47**
Albumins, g/l	52.2±3.21	44.2±1.57*	39.3±1.65**
Total cholesterol, mmol/L	4.17±1.11	5.7±0.97	6.3±1.12
Urea, mmol/L	6.3±0.19	7.1±0.24*	14.3±1.15***ˆˆˆ
Urea after dialysis	-	-	7.8±0.23*
Creatinine, mcmol/l	74.8±3.53	103.1±5.3	792.2±40.4***ˆˆˆ
Creatinine after dialysis	-	-	178.2±8.41***ˆˆˆ
Body mass index, kg/m²	23.7±1.19	28.7±1.14***	27.1±1.07**
Systolic blood pressure, millimeter of mercury	124.2±4.68	154.3±7.41*	151.3±6.33*
Diastolic blood pressure, millimeter of mercury	81.4±2.52	98.2±4.79*	96.4±5.13*

Note. * - p<0.05; ** - p<0.01; *** - p<0.001; in comparison with the control group. ˆ - p<0.05; ˆˆ - p<0.01; ˆˆˆ - p<0.001; in comparison between Group I and Group II.

The significant decrease in albumin in the two research groups comes under notice. Indices of nitrogen metabolism were within normal range in patients of Group I whereas a significant increase in these data was observed in the group of dialysis patients even after adequate HD session (p<0.001).

In order to define vascular endothelium functions we studied the levels of ET-1. Many scientists consider its increased concentration to be associated with ED [7, 11]. The levels of CRP and TNF-alpha were determined in all patients of Group I and Group II (Table 2). The obtained results implied that the levels of ET-1 and inflammatory markers were significantly higher in patients of Group II in comparison with the control group and the comparison group (p<0.001 and p<0.001). We consider the increase in these markers in patients on HD to be caused by chronic inflammation and uraemic intoxication.

Table 2

The level of CRP, ET-1 and TNF-alpha in blood serum in the control group and comparison group

Parameters	Control n=20	Group I (AH with CHF) n=42	Group II (V stage CKD, on HD with CHF) n=46
TNF-alpha, pg/ml	23.6±3.62	63.2±12.29***	103.5±25.61***ˆˆˆ
ET-1, pg/ml	5.3±0.32	9.7±1.37**	12.01±1.36***ˆˆˆ
CRP, mg/l	6 [0;6]	9.0 [3.0;24]**	24.0 [12;24]***ˆˆˆ

Note. * - p<0.05; ** - p<0.01; *** - p<0.001; in comparison with the control group. ˆ - p<0.05; ˆˆ - p<0.01; ˆˆˆ - p<0.001; in comparison between Group I and Group II.

The difference between the studied parameters is clearly presented in the form of box plot (Fig. 1-3). Increase in inflammatory mediators and endothelial vasoconstrictor factor in both groups of patients may indicate may indicate a significant part of these biological substances in CHF development and progression [4,5,13].

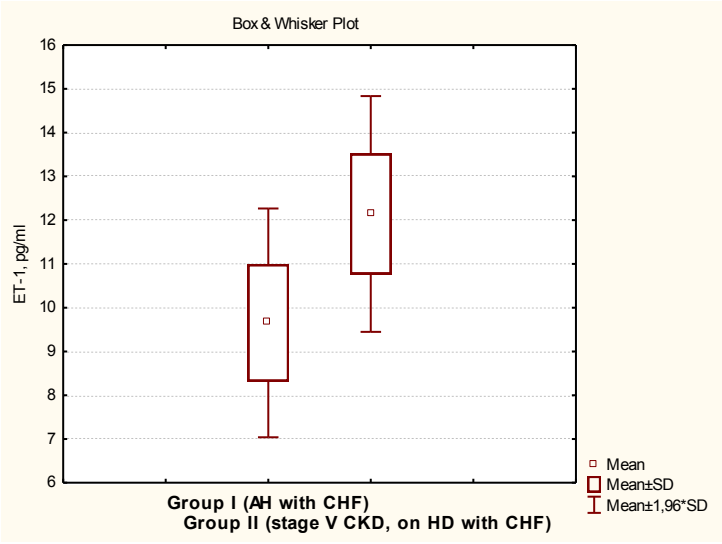

Fig. 1. Box plot of ET-1 level in examined groups of patients with CHF

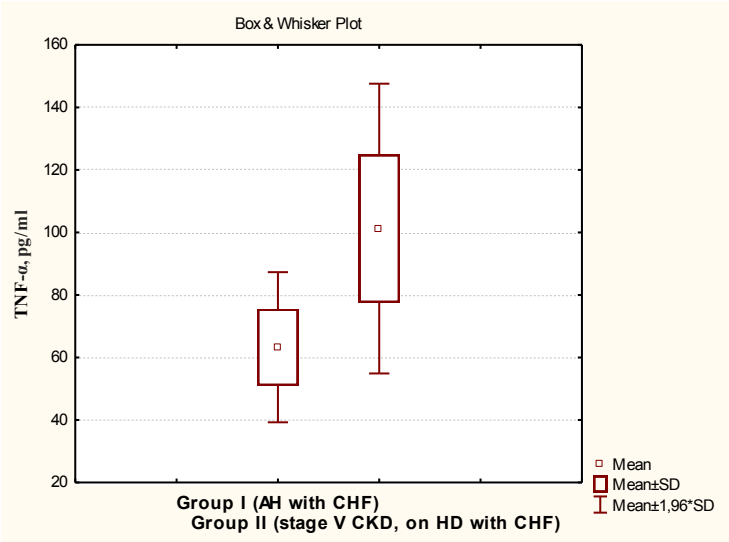

Fig.2. Box plot of TNF-α level in examined groups of patients with CHF

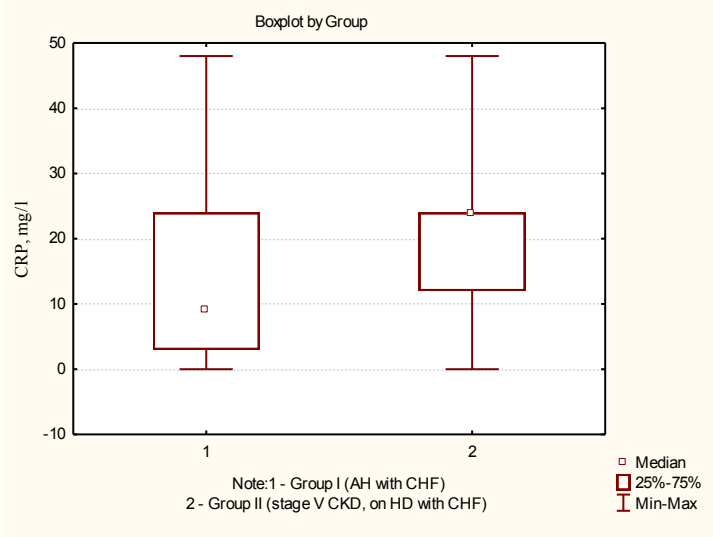

Fig.3. Box plot of CRP level in examined groups of patients with CHF

Literature data provide information about the relationship of ET-1 with CHF functional class [3, 9]. However, as it follows from our research, when CHF functional class was the same in patients

of both research groups, this marker was significantly higher in patients on HD being apparently associated with more severe ED in this cohort of examined patients.

Recent studies have found a relation between the level of TNF-alpha and CHF stage, but such studies usually do not include patients who require replacement therapy [4]. However, analyzing the data obtained in our research, the levels of these inflammatory markers in patients on HD were much higher than in patients with preserved renal function, in spite of the same functional class of CHF (Fig. 2).

High level of CRP in the group of dialysis patients may be provoked by chronic inflammation, which is an essential sign in case of the terminal uremia [1].

Having analyzed the correlation matrix, a direct moderate correlation between the level of TNF-alpha and CRP in both research groups, but this correlation was much stronger in the group of dialysis patients (r=0.52; p<0.05 and r=0.65; p<0.05, respectively) (Fig.4 and Fig.5). The obtained results are in agreement with the literature data concerning the relationship of these inflammatory markers [12,14].

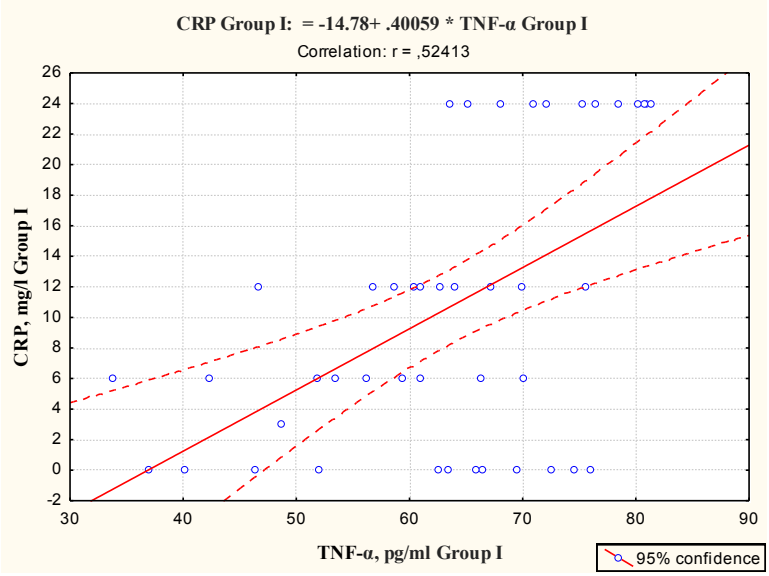

Fig.4. Correlation between CRP and TNF-α level in patients of Group I

Increase in CRP and TNF-alpha is well known to be an independent predictor of cardiovascular events [4]. Correlation analysis between these markers and ET-1 level was performed to study the value of these indices in the development of ED (Fig. 6 – Fig 9).

Direct moderate correlation between TNF-alpha and ET-1 (Fig. 8) (r = 0.48; p<0.05) and between CRP and ET-1 (Fig. 6) (r = 0.56, p<0.05) was established in Group I. In Group II, the relation between TNF-alpha and ET-1 (Fig. 9) was significantly stronger (r=0.58; p<0.05); between the levels of CRP and ET-1(Fig. 7) it was weaker (r=0.37, p<0.05).

Established correlation between the level of TNF-alpha and ET-1 in both study groups indicates that the simultaneous increase in these markers is a significant predictor of ED and cardiovascular complications in patients with CHF regardless of renal function. Since ET-1 causes endothelium-dependent vasoconstriction, vessel constriction provokes the synthesis of TNF-alpha which affects the increase of ET-1indirectly through CRP. Thus, a “vicious circle” of pathogenic changes is formed leading to a disease progression. At the same time, though increase in CRP level in the blood of patients on HD is indisputable factor of CHF progression, it may predict ED development to a lesser extent in this population.

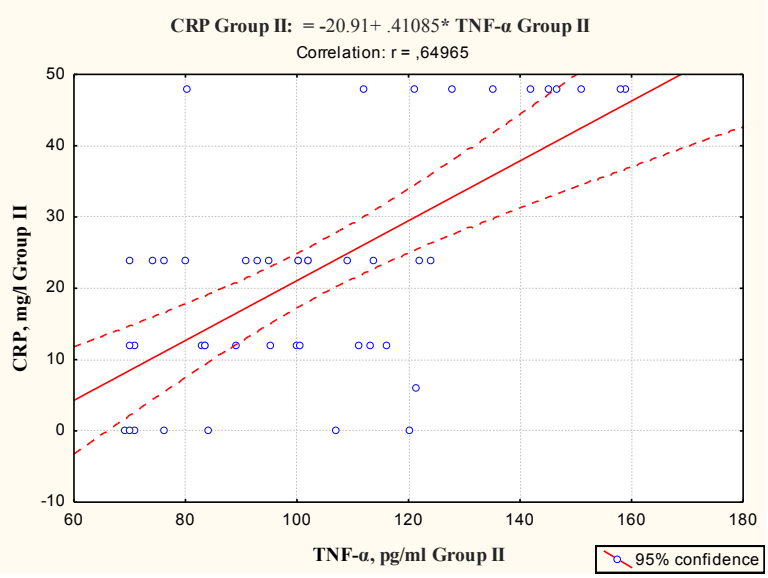

Fig. 5. Correlation between CRP and TNF-α level in patients of Group II

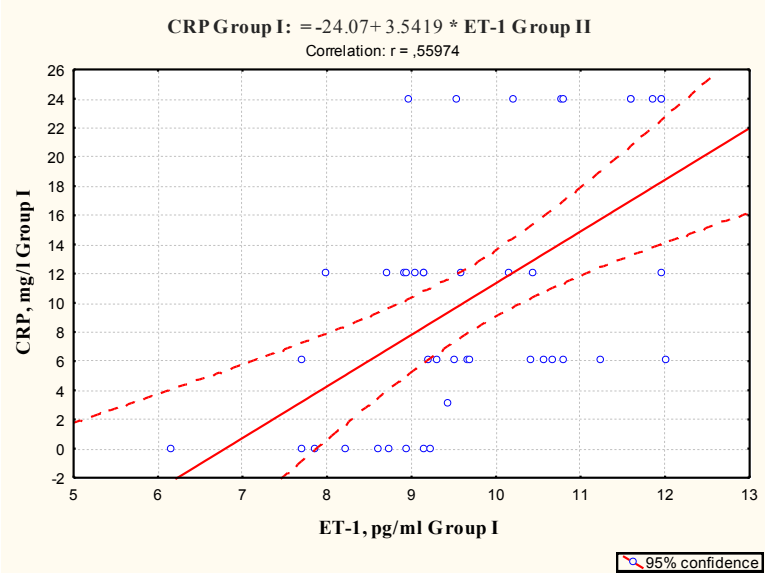

Fig. 6. Correlation between CRP and ET-1 level in patients of Group I

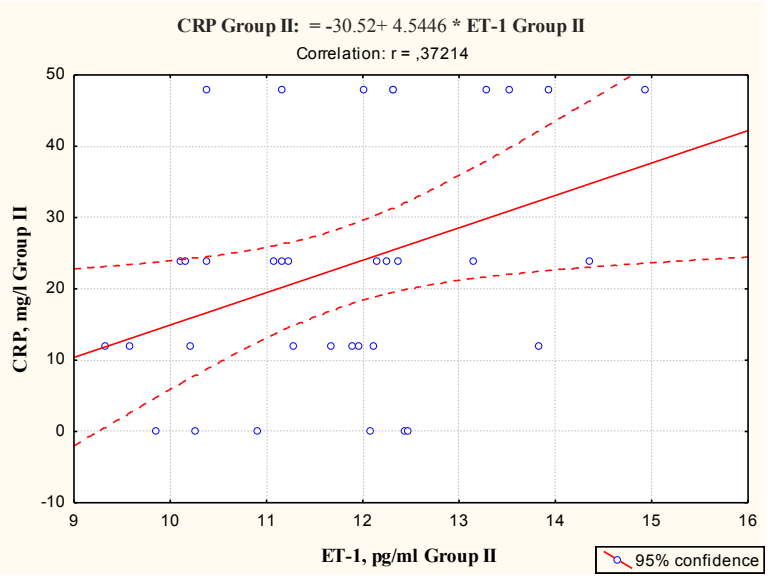

Fig. 7. Correlation between CRP and ET-1 level in patients of Group II

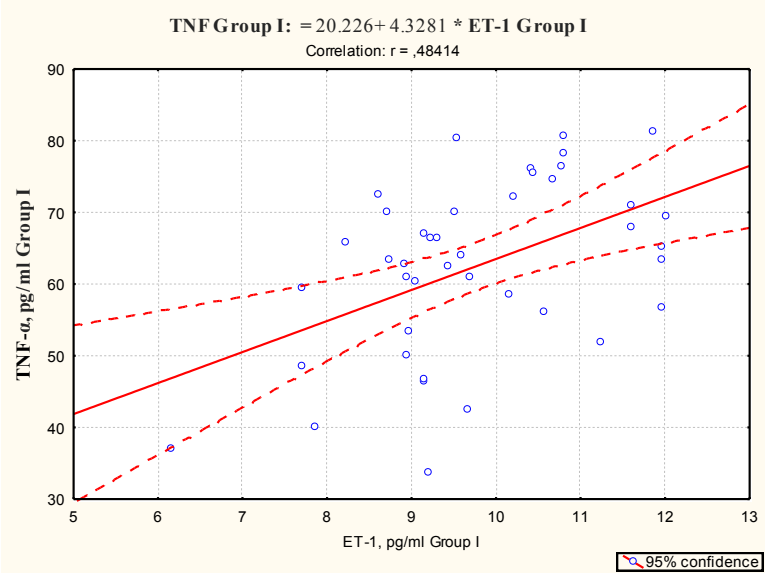

Fig.8. Correlation between TNF-α and ET-1 level in patients of Group I

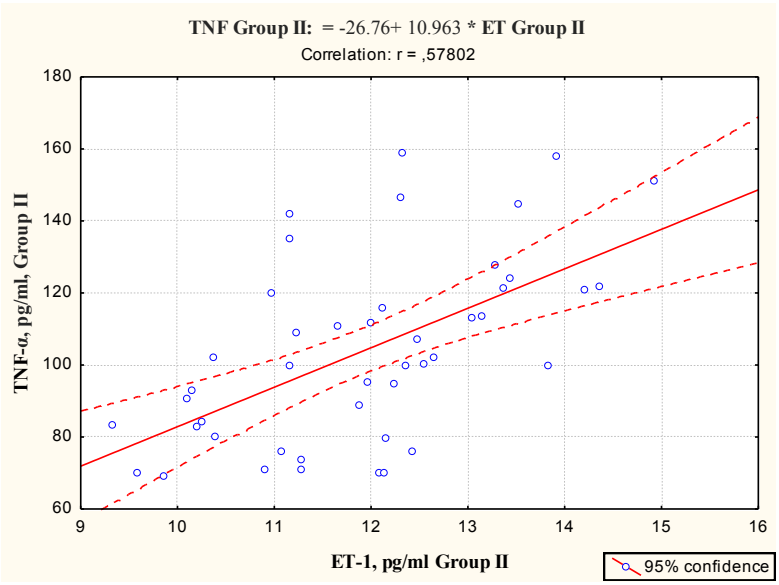

Fig.9. Correlation between TNF-α and ET-1 level in patients of Group II

Conclusions

1. CRP levels of TNF-alpha, ET-1 in patients on HD were found to be significantly different from the same data in patients with preserved renal function, which is clearly associated with more pronounced signs of inflammation and ED in a cohort of dialysis patients.
2. The impact of TNF-± in the development of ED in both groups was proved.
3. Despite the higher level of CRP in patients with stage V CKD being corrected by HD with CHF, this biomarker had less impact on the prognosis of ED than in the general population of patients with CHF.

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