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Inflammatory Markers and Procoagulants in Chronic Renal Disease Stages 1-4

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ABSTRACT

Aim: Introduction: Starting from the point that the chronic kidney disease (CKD) is chronic, inflammatory and hypercoagulable state characterized by an increase in procoagulant and inflammatory markers high cardiovascular morbidity and mortality in these patients could be explained. Aim: The aim of the research was to monitor inflammatory markers and procoagulants in various stages of kidney disease (stage 1-4). **Materials and Methods:** The research included 120 subjects older than 18 years with CKD stages 1-4 examined and monitored in Clinic of Nephrology, University Clinical Centre Sarajevo over a period of 24 months. The research included determining the following laboratory parameters: serum creatinine, serum albumin, C-reactive protein, leukocytes in the blood, plasma fibrinogen, D-dimer, antithrombin III, coagulation factors VII (FC VII) and coagulation factor VIII (FC VIII). **Results:** With the progression of kidney disease (CKD stages 1-4), there was a significant increase of inflammatory and procoagulant markers: CRP, fibrinogen and coagulation factor VIII, and an increase in the average values of leukocytes and a reduction in the value of antithrombin III, but without statistical significance. Also, there were no significant differences in the values of D-dimer and coagulation factor VII.

Conclusion: The progression of kidney disease is significantly associated with inflammation, which could in the future be useful in prognostic and therapeutic purposes. Connection of CKD with inflammation and proven connection of inflammation with cardiovascular risk indicates the potential value of some biomarkers, which could in the future identify as predictors of outcome and could have the benefit in the early diagnosis and treatment of cardiovascular disease in CKD.

Key words: inflammatory biomarkers, procoagulants, cystatin C, cardiovascular morbidity

1. INTRODUCTION

Starting from the premise that the chronic kidney disease (CKD) chronic, inflammatory and hypercoagulable state characterized by an increase in procoagulant and inflammatory markers high cardiovascular (CV) morbidity and mortality in these patients could be explained. Traditional CV factors could not fully explain the increased CV risk. Non-traditional CV risk factors include, among others: albuminuria, lipoprotein disorder, lipoprotein

A and apo-A isoform, anemia, disorder of mineral metabolism, activation of oxidative stress/in-

flammatory markers, malnutrition, thrombogenic factors, changed balance of nitric oxide/endothelin. Some of these factors, such as anemia, metabolic disbalance and inflammation are present in patients with CKD (1).

Researches show that inflammation markers can predict the decline in kidney function. Measurement of parameters of thrombogenesis, thrombin-antithrombin III complex (TAT), prothrombin fragment (F1), D-dimer, fibrinopeptide A (FP-A) and inflammation - CD 40, myeloperoxidase (MPO), tumor necrosis factor alpha (TNF-alpha), monocyte

chemotactic protein-1 (MCP-1) and nitric oxide (NO) in one of the studies shows that patients with ESRD (end-stage renal disease) are exposed to activity of coagulant and inflammatory processes (2).

Different biomarkers in this regard could have a role in the identification of patients at risk of CV disease development (3).

Although the mechanisms are not fully understood, it is believed that the increased risk of VTE in CKD occurs as a result of haemostatic disorder, including procoagulant activation, reduction of endogenous anticoagulants, disorder activation and aggregation of platelets and decrease in fibrinolytic activity. Pathogenesis varies depending on the cause of CKD. Uremia is associated with prolonged bleeding time, impaired adhesion and aggregation of platelets due to a series of extrinsic and intrinsic factors. It is well known role of increase of coagulation factor VIII - Von Willebrand's factor, and deficiency of antithrombin III, protein C and protein S. (4)

MATERIAL AND METHODS

This prospective study included 120 patients older than 18 years, examined and monitored over a period of 24 months on Clinic of Nephrology, University Clinical Center Sarajevo. Thirty patients in every stage of CKD from 1-4 were included, wherein the stages of CKD are determined according to KDIGO (International Kidney Disease: Improving Global Outcomes) classification (5): 30 patients in stage 1 of CKD, which is characterized by glomerular filtration rate > 90 ml/min/1.73 m² and the presence of albuminuria, proteinuria or pathological urine sediment; 30 patients in stage 2 of CKD, which is characterized by mildly decreased glomerular filtration rate of 60-89 ml/min/1.73 m² and the presence of albuminuria, proteinuria or pathological urine sediment; 30 patients in stage 3 of CKD with moderate reduction of glomerular filtration rate of 30-59 ml/min/ 1.73 m² and 30 in stage 4 (progressed) of CKD with a glomerular filtration rate of 15-29 ml/min/1.73m².

The research did not include: patients with acute renal failure, patients with kidney transplant, heavier mental state, patients with severe liver failure and liver cirrhosis, with malignant disease, severe infection and sepsis, with primary hematologic disease, type I diabetes, pregnant and lactating women. In the methodological approach in this research we used personal, demographic and anamnesis data, physical examination and methods of laboratory diagnosis.

The research included determining the following laboratory parameters: serum creatinine, serum albumin and C-reactive protein, leukocytes in the blood, plasma fibrinogen, D-dimer, antithrombin III, coagulation factors VII (FC VII) and VIII (FC VIII).

The study results were statistically analyzed by using descriptive statistics and Student- t test, with the acceptance of statistical significance at $p < 0.05$. For determining normal distribution of the examined variables was used Kolmogorov-Smirnov test. The statistical differences in mean values between the examined groups was determined by using ANOVA method with Turkeys post

hoc test for variables with normal distribution or Kruskal-Wallis and Mann-Whitney post hoc test for variables that do not have a normal distribution.

2. RESULTS

Analysis of the average values of leukocytes shows that the patients in group IV had the highest average values of 7.97 ± 1.8 (range from 4.83 - 12.90), and patients in group I had the lowest 7.2 ± 2.4 (range 3.90 - 14.03). The average value of leukocytes in blood is gradually increased as seen from group I to group IV. Analysis of variance and Turkey's post hoc test indicates that there is no statistically significant difference between examined groups $p > 0.05$ ($F = 0.811$; $p = 0.490$). Statistical analysis of the average values of CRP shows that the patients in group II had the highest value of 10.07 ± 13.02 (range 0.5 - 66.6), while patients in group I had the lowest 2.77 ± 3.70 (range 0.00 - 16.60). Analysis of variance and Turkey's post hoc test shows that there is a statistically significant difference $p < 0.05$ in the average values of CRP ($F = 2.787$; $p = 0.044$). The average value of CRP in group II is unexpectedly higher in comparison to the group III, but that difference was not statistically significant, $p = 0.115$. Analysis of the average values of fibrinogen through Mann-Whitney test showed statistically significant differences at level $p < 0.05$ (KW $\chi^2 = 8.643$; $p = 0.032$). The patients in group IV had the highest value of 4.68 ± 1.4 (range 2.3 to 8.0), while patients in group I had the lowest 3.74 ± 1.3 (range 1.8 - 6.1). As in the case of leukocytes (as opposed to CRP) we record a linear increase of average values of fibrinogen observed from group I to group IV of patients. Patients in group I had the highest average value of serum albumin 39.17 ± 4.1 (range 29.0 - 47.0), and patients in group II had the lowest 32.73 ± 9.88 (range 12.0 - 56.0). Analysis of the average values of serum albumin through analysis of variance and Turkey's post hoc test indicates that there is a statistically significant difference at level $p < 0.01$ ($F = 4.772$; $p = 0.004$). A quite uneven ratio of the average values of serum albumin in the observed groups was detected. The patients in group II had the highest average value of D-dimer 2.27 ± 4.13 (range 0,17 - 21,92), while patients in group I had the lowest 0.65 ± 0.71 (range 0.17 - 3.09). Analysis of average values of D-dimer through analysis of variance and Turkey's post hoc test shows that there was no significant difference $p > 0.05$ ($F = 2.577$; $p = 0.057$). The average value of D-dimer in the group I is lower than in the other groups. Although in the total sample there is no statistically significant difference, $p = 0.057$ (close to statistical significance), there is statistically significant difference between the group I and all other groups of patients (between I and II, $p = 0.04$, $t = -2.116$; between I and III, $p = 0.01$, $t = -2.651$; and between I and IV; $p = 0.002$, $t = -3.172$). Patients in group I had the highest average value of antitrombin III 100.01 ± 13.1 (range 64.2 - 124.1), and patients in group IV had the lowest 93.27 ± 20.60 (range 38.2 - 122.6). Analysis of variance and Turkey's post hoc test shows that there was no significant difference $p > 0.05$ ($F = 0.861$; $p = 0.464$). There is a slight linear decrease of the average values of antithrombin III

from group I to group IV. Neither group in comparison to the other shows statistically significant difference in the average values of antithrombin III ($p > 0.05$). The highest average value of FC VII 136.57 ± 37.26 (range 59.7 - 210.6) had patients in group II, and the lowest 115.97 ± 47.31 (range 39.6 - 210.0) patients in group III. Statistical analysis of the average values of FC VII through Mann-Whitney test showed that there was no significant difference $p > 0.05$ (KW $\chi^2 = 1.162$; $p = 0.327$). It is quite uneven ratio of average values of FC VII observed from group I to group IV of patients. Patients in group III had the highest average value of FC VIII 162.5 ± 27.8 (range from 120.3 - 212.3), while patients in group I had the lowest 125.5 ± 35.3 (range 55.2 - 207). Analysis of average values of FC VIII through Mann-Whitney test shows that there is a statistically significant difference at level $p < 0.01$ (KW $\chi^2 = 22.291$, $p = 0.0001$). We see quite steady increase in the average values of FC VIII from group I to group III of patients and consistent average values between the groups III and IV (Table 1).

parameters	AM	SD	SEM	95% confidence interval		p
				Lower	Upper	
Cystatin C mg/l	1.614	0.834	0.076	1.463	1.765	0.0001
Leukocytes x10 ⁹	7.659	2.247	0.205	7.253	8.065	0.490
CRP mg/l	6.329	10.078	0.920	4.508	8.151	0.044
Fibrinogen g/l	4.363	1.409	0.128	4.108	4.617	0.032
Albumins g/l	36.510	7.477	0.683	35.16	37.86	0.004
D-dimer mg/l	1.623	2.588	0.236	1.155	2.091	0.057
Antithrombin III mg/l	96.895	16.329	1.490	93.943	99.847	0.464
FC VII %	127.620	43.663	3.985	119.727	135.513	0.327
FC VIII %	146.259	36.307	3.314	139.696	152.822	0.0001

Table 1. Analysis of the average values of inflammatory parameters and procoagulants in the total sample Notes: AM- arithmetic mean, SD- standard deviation, SEM- standard error of the mean, 95% C- confidence interval, p- statistically significant difference in the average values of the parameters observed

3. DISCUSSION

The Inflammation and chronic kidney disease are associated with the cardiovascular diseases (6). In the general population, inflammation is one firmly established risk marker of cardiovascular morbidity, where CRP and leukocytosis are independently associated with adverse cardiovascular outcome.

Connection of inflammation with renal impairment and increased cardiovascular morbidity and mortality is a significant subject of research of many authors. Earlier researches have shown an association of CKD with increased values of CRP, as well as the connection of CRP with cardiovascular risk in patients with ESRD. It remains to examine the ratio of CV risk and inflammation in patients with milder level of renal impairment (7). Other studies indicate that CRP and endothelial dysfunction could provide prognostic information about cardiovascular disorders in kidney patients. One of them monitored importance of chronic inflammation, oxidative stress and endothelial dysfunction in cardiovascular disease in kidney patients. It demonstrated that patients with higher CRP had significantly lower eGFR and serum albumins and increased lipid hydroxy peroxidasis, while endothelial function was associated with intracellular oxidative stress in patients with chronic kidney disease (8). And one recent study has shown that inflammation, assessed by measuring the levels of CRP correlates with endothelial dysfunction and atherosclerotic changes in patients with CKD (9).

It has been proven in numerous studies that different thrombogenic and inflammatory cardiovascular risk factors are significantly increased in CKD. One of the studies in a larger group of CKD patients (10), which fol-

lowed connection between albuminuria, kidney function and inflammatory biomarkers, found a higher level of IL-1 β , IL-1RA, IL-6, TNF- α , TGF- β , CRP and fibrinogen among patients with lower eGFR. It was something that our study in a population of CKD patients from stages 1-4, except in the case of CRP and fibrinogen, has shown and in the case of coagulation factor VIII. Serum fibrinogen is a predictor of CV morbidity in the general population and independent predictor of CV and all causes of morbidity in ESRD. Studies indicate that increased levels of fibrinogen can predict mortality with patients in stages 3 and 4 of CKD (11).

The linear increase in the average values of leukocytes from group I to group IV of patients was observed, but it was not statistically significant. With progression of kidney disease it is recorded a gradual decline in the average values of antithrombin III, although the same, too, was not statistically significant. There were no differences in the values of FC VII, as in I and II groups of patients, thus in other stages of kidney disease (with eGFR < 60 ml / min / 1.73m²). Unlike FC VII whose average values are rather uneven observed from I-IV group, with the progression of kidney disease a gradual, statistically significant, linear increase in the average value of FC VIII is monitored.

Several studies have compared the level of procoagulant and inflammatory markers in patients with and without CKD. Generally, in these studies, patients with ESRD, nephrotic syndrome, and the initial stages of CKD had increased level of CRP, fibrinogen, D-dimer, FC VII and FC VIII, which is explained by a higher synthesis in comparison to losses in urine, although accurate mechanisms are not yet known (12). There is not

enough convincing evidence about the role of deficiency of antithrombin III, protein S and protein C at increased risk of VTE (13). The average value of serum albumin in our research shows, although significant, quite uneven differences between groups (the lowest in II group of patients), which could be a result of the influence of different factors, for example. Chronic inflammation, nutritional status and protein loss in urine.

The importance of antithrombin III in increasing the risk of VTE in patients with CKD is not completely convincing. Not all so far published studies confirmed the correlation between the deficit of antithrombin III in nephrotic patients and the risk of VTE.

4. CONCLUSION

With the progression of kidney disease (CKD stages 1-4) it comes to significantly increase of inflammatory and procoagulant markers of CRP, fibrinogen and coagulation factor VIII, and an increase in the average values of leukocytes and falling of antithrombin III, but without statistical significance. Connection of CKD with inflammation and proven connection of inflammation with CV risk indicates the potential diagnostic value of some biomarkers, which could open up possibilities for new therapeutic alternatives. Various biomarkers could in the future identify as predictors of outcome and could have the benefit in the early diagnosis and treatment of CVD in CKD.

CONFLICT OF INTEREST: NONE DECLARED.

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