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CYSTATIN C LEVELS, STATUS OF OXIDANT-ANTIOXIDANT AND INFLAMMATION IN HEMODIALYSIS PATIENTS

HEMODİALİZ HASTALARINDA SİSTATİN C DÜZEYLERİ, OKSİDAN-ANTİOKSİDAN VE İNFLAMASYON DURUMU

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Abstract

Objective: Cystatin C is considered an important marker for the detection of renal dysfunction. It was aimed to determine cystatin C levels, status of oxidant-antioxidant and inflammation in hemodialysis (HD) patients.
Methods: The study groups consisted of 20 HD patients and 20 healthy controls. Blood samples were obtained from the control group and from the HD group before hemodialysis. Serum cystatin C, total antioxidant capacity (TAC), total oxidative state (TOS), tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6) interleukin 18 (IL-18) levels were detected by Enzyme Linked Immunosorbent Assay (ELISA), C-reactive protein (CRP) and some biochemical parameters were analyzed by autoanalyser.
Results: Cystatin C, TOS, TNF-α, IL-6, IL-18 and CRP levels of the HD group were significantly higher than the control group.
Conclusion: As a result; oxidative stress and inflammation were increased in patients with chronic renal failure undergoing hemodialysis. New strategies and new studies are needed to reduce the increase in oxidative stress and inflammation in HD patients.

Keywords: *Hemodialysis patients, cystatin C, oxidative stress, inflammation.*

Öz

Amaç: Sistatin C, böbrek fonksiyon bozukluğunun saptanması için önemli bir belirteç olarak kabul edilir. Hemodiyaliz (HD) hastalarında sistatin C düzeylerinin, oksidan-antioksidan ve inflamasyon durumunun belirlenmesi amaçlandı.
Yöntem: Çalışma grupları 20 HD hastası ve 20 sağlıklı kontrolden oluşturuldu. Hemodiyaliz öncesi kontrol grubundan ve HD grubundan kan örnekleri alındı. Serum sistatin C, total antioksidan kapasite (TAC), total oksidatif durum (TOS), tümör nekroz faktör alfa (TNF-α), interlökin 6 (IL-6), interlökin 18 (IL-18) seviyeleri ELISA ile C- reaktif protein (CRP) ve bazı biyokimyasal parametreler ise otoanalizörde analiz edildi.
Bulgular: HD grubunun sistatin C, TOS, TNF-α, IL-6, IL-18 ve CRP düzeyleri kontrol grubuna göre anlamlı derecede yüksekti.
Sonuç: Sonuç olarak; hemodiyaliz uygulanan kronik böbrek yetmezliği olan hastalarda oksidatif stres ve inflamasyon artmıştır. Hemodiyaliz hastalarında oksidatif stres ve inflamasyonu azaltmak için yeni stratejilere ve yeni çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: *Hemodiyaliz hastaları, sistatin C, oksidatif stres, inflamasyon.*

Introduction

Cystatin C is a cysteine proteinase inhibitor and has low molecular weight. It is synthesized in all nucleated cells in the body. Due to its small molecular structure and basic isoelectric pH, it is more freely filtered from glomeruli than other proteins.[1] Cystatin C is a good biomarker of glomerular filtration rate (GFR), such as serum creatinine and creatinine clearance. It has some advantages since its excretion is only through glomerular filtration and does not change with age, gender and muscle.[2-3]

The excessive accumulation of nitrogen, the final product of protein and amino acid metabolism, in the blood is defined as uremia (azotemia). In healthy individuals, uremic metabolites or toxins are removed from the body by the kidneys. However, in patients with chronic kidney disease, clearance is impaired, excretion cannot be performed and therefore the amount of urea in the blood increases.[4] In chronic kidney failure, uremic metabolites cause the progression of oxidative stress and inflammation.[5-7.]

Oxidative stress is defined as the disruption of oxidative balance as a result of the lack of antioxidants and increased reactive oxygen species (ROS) such as hydroxyl radical (˙OH), superoxide radical and hydrogen peroxide which occurs during cellular metabolism. This increase in the amount of ROS ultimately causes damage to cell membranes, disruption in the structure and function of intracellular proteins, and structural damage in DNA, leading to cell injury.[7] They are produced depending on causes such as ischemia-reperfusion, aging, radiation, high oxygen pressure, inflammation and exposure to chemical agents. Oxidative stress is responsible for the pathogenesis of many diseases, especially cancer, diabetes, cardiovascular and neurological diseases, atherosclerosis and inflammatory disorders.[5] It is found even in the early stages of kidney diseases, gradually increases with kidney failure and gets worse with hemodialysis applications.[8]

Under normal physiological conditions, inflammation is a protective response to various harmful stimuli. Uremia is a proinflammatory condition. Dialysis therapy itself can contribute to chronic inflammation due to blood exposure to the dialysate membrane, exposure to endotoxins.[9] Inflammation increases in direct proportion with the progression of the disease, from the initial stages of chronic kidney disease to the final stage. However, in some debilitating chronic diseases like chronic renal disease, inflammation becomes uncontrolled and permanent. Inflammatory markers such as CRP, IL-6 and TNF-α, IL-18 are used effectively to evaluate inflammation.[10]

With this study, especially cystatin C and TAC, TOS, CRP, IL-6, TNF-α, and IL-18 levels will be determined in hemodialysis patients.

Methods

Evaluation of Patients and Forming Groups

The material of this study was 30 to 85 years-old 20 volunteer patients who were dialyzed in the HD at District State Hospital 3 times a week (Van Yuzuncu Yıl University Non-Interventional Clinical Research Ethics Committee, Approval No: 2019/09-05). Seven of the patients were female and 13 were male. Patients with HD duration less than one year, patients with known malignancy, active infection and diabetic patients were excluded from the study. HD duration, age, gender, height, body weight and body mass indexes of the patients were recorded. A control group of healthy volunteers was created for the study. Individuals whose age and gender characteristics of the control group were similar to the patient group (13 men, 7 women). The data of the study were collected between August and September 2019.

Taking Blood Samples and Biochemical Analysis

Blood samples were taken from the HD group included in the study after 12 hours of fasting, before dialysis, and the healthy control group after 12 hours of fasting. The blood samples were centrifuged and sera were separated. Analyzes of some biochemical parameters (serum urea, creatinine, glucose, CRP, total protein, albumin, cholesterol, triglyceride, VLDL, LDL, HDL, CK-MB, troponin, sodium, potassium, chlorine, calcium, iron) were done immediately by the autoanalyzer (Dimension RxL, Advia Centaur CP, Siemens, Germany). Serum samples stored in the freezer (-20°C) until the day of analysis. Cystatin C (Bioassay Technology Laboratory, Cat. No. E1104Hu, China) TAC (Rel Assay Diagnostics, Cat. No. RL0017, Turkey), TOS (Rel Assay Diagnostics, Cat. No. RL0024, Turkey), TNF-α (Bioassay Technology Laboratory, Cat. No. E0032Hu, China), IL-6 (Bioassay Technology Laboratory, Cat. No. E0090Hu, China), IL-18 (Bioassay Technology Laboratory, Cat. No. E014Hu, China) levels were determined by ELISA.

Statistical Analysis

Descriptive statistics for continuous variables from the features mentioned; while expressed as Average and Standard Deviation, it was expressed as numbers and percentages for categorical variables. One-Way Variance Analysis was performed to compare group averages in terms of continuous variables. In calculations, statistical significance level was taken as 5% and SPSS statistical software was used.

Results

A total of 40 people, 20 healthy control groups and 20 HD patients were included in the study. Gender distributions are the control group; 13 men (65%), 7 women (35%), HD group; it was 13 males (65%), 7 females (35%). Ages, body weights, heights, body mass index in the control and HD group and hemodialysis time of HD patients were given in Table 1. The hemodialysis time of the patients in the HD group was 4.30 ± 1.84 years. There were patients who had undergone hemodialysis for at least 3 and at most 11 years. The average of the biochemical analysis of the control and HD groups were also shown in Table 1.

The mean values of cystatin C, TAC, TOS, TNF-α, IL-6, IL18 levels of the control and HD group are given in Table 2. Accordingly, cystatin C, TOS, TNF-α, IL-6, IL-18 and CRP levels of the HD group were found to be statistically significantly higher than the control group ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.05$, $p<0.05$, respectively).

Table 1. Demographic data and laboratory tests of the groups

	Control (n: 20) Mean ± SD	HD Group (n: 20) Mean± SD	*p*
Duration of hemodialysis (year)		4.30 ± 1.84	
Sex (M/F)	13/7	13/7	
Age (years)	51.35 ± 14.74	58.78 ± 11.71	0.096
Body weight (kg)	72.10 ± 9.69	56.55 ± 6.26	0.001
Length (m)	1.73 ± 0.06	1.68 ± 0.06	0.022
BMI (kg/m^2)	24.08 ± 2.54	19.97 ± 1.60	0.001
Laboratory tests			
Urea (mg/dl)	33.05 ± 7.83	104.52 ± 26.83	0.001
Creatinine (mg/dl)	0.98 ± 0.45	9.12 ± 2.89	0.001
Glucose (mg/dl)	100.95 ± 14.29	92.90 ± 21.76	0.175
T. Protein (g/dl)	7.41 ± 0.36	6.87 ± 0.43	0.001
Albumin (g/dl)	3.71 ± 0.44	3.17 ± 0.37	0.001
Triglyceride (mg/dl)	113.25 ± 40.56	127.65 ± 67.20	0.417
Cholesterol (mg/dl)	161.80 ± 28.91	143.45 ± 31.26	0.061
LDL (mg/dl)	95.86 ± 35.53	83.63 ± 23.77	0.208
HDL (mg/dl)	40.85 ± 7.86	38.96 ± 16.71	0.650
VLDL (mg/dl)	32.570 ± 25.60	23.00 ± 10.42	0.130
CK-MB (U/L)	16.65 ± 5.72	11.65 ± 6.02	0.011
Troponin (ng/ml)	0.000 ± 0.000	0.012 ± 0.011	0.001
Sodium (mmol/L)	143.15 ± 3.30	140.25 ± 2.59	0.004
Potassium (mmol/L)	4.18 ± 0.36	4.93 ± 0.65	0.001
Chlorine (mmol/L)	104.10 ± 1.7	102.40 ± 2.37	0.013
Calcium (mg/dl)	9.26 ± 0.39	8.95 ± 0.60	0.064
Iron (µg/dl)	70.95 ± 34.46	58.15 ± 21.61	0.167

Table 2. Cystatin C, TAC, TOS, TNF-α, IL-6, IL18 and CRP levels of the groups

	Control (n: 20) Mean ± SD	HD Group (n: 20) Mean± SD	*p*
Cystatin C (ng/ml)	0.72 ± 0.22	3.09 ± 0.40	0.001
TAC (mmol Trolox Equiv/L)	1.09 ± 0.11	1.02 ± 0.13	0.110
TOS (µmol H2O2 Equiv/L)	2.24 ± 1.47	7.48 ± 5.59	0.001
TNF-α (ng/L)	5.94 ±1.35	17.64 ± 3.62	0.001
IL-6 (ng/L)	6.92 ± 2.04	10.46 ± 2.87	0.001
IL-18 (ng/L)	39.94 ± 15.72	52.06 ± 15.85	0.020
CRP (mg/L)	3.99 ± 0.70	8.50 ± 9.08	0.033

Discussion

Cystatin C is a good biomarker of GFR, such as serum creatinine and creatinine clearance. It is generally known as a kidney function test better than serum creatinine in the elderly and people with moderate renal dysfunction.[11,12] However, serum cystatin C does not appear to be superior to serum creatinine in patients with acute renal failure, acute allograft dysfunction and chronic renal failure.[13,14] In addition, it may not be suitable for monitoring patients who undergo peritoneal dialysis.[15] Although this protein is initially believed to be independent of non-renal factors, in recent years, it has been shown that cystatin C levels may be associated with different clinical and demographic conditions.[11,16,17] These include age, gender, body type, lipoprotein anomalies, presence of metabolic syndrome, inflammation, arterial hypertension, and other factors. All these factors have been associated with serum cystatin C levels in the general population.[18-21] On the contrary, the relation of extrarenal factors with cystatin C levels in pre-dialysis patients with chronic renal disease is not understood.[22] They measured cystatin C levels in a total of 52 patients with chronic kidney disease, 22 in the 3rd stage, 25 in the 4th stage, 5 in the 5th stage who did not undergo dialysis, and determined 2.35 mg/L. They reported that cystatin C levels are closely related to kidney function parameters in advanced chronic kidney disease.[22] Serum cystatin C level was found to be 1.90 mg/L in various kidney patients and 7.14 mg/L in dialysis patients.[2] In this study, cystatin C level was found to be 3.09 ng/ml in HD patients and 0.72 ng/ml in the control group. According to the findings, cystatin C level was found to be high in HD patients.

There are many studies showing that oxidative stress increases and antioxidants decrease in chronic kidney patients.[23-27] It is even reported that hemodialysis contributed to oxidative stress.[28] It has been suggested that total antioxidant activity decreases significantly in hemodialysis patients and this defective antioxidant activity may contribute to uremic toxicity through peroxidative cell damage.[29] It is stated that the serum antioxidant capacity before dialysis increases significantly in patients with chronic kidney failure and who are dialyzed, and this is due to the increased uric acid level.[30] There are also studies reporting that total antioxidant capacity decreased[30] or no change after hemodialysis.[23,31]

The assessment of general oxidative stress causes many problems due to the diversity of natural antioxidant enzymes, non-enzymatic free radical scavengers and the presence of ROS. Separate measurements of individual antioxidant enzymes, antioxidant molecules, and different oxidants are laborious and expensive due to the wide variety of parameters of the oxidant-antioxidant system. This greatly hinders the assessment of the total oxidant-antioxidant balance. TOS parameter, which covers a wide range of oxidants that play a role, may be more useful. On the other hand, the evaluation of antioxidant status with the non-enzymatic component can be done by measuring the TAC. Analysis of parameters such as TAC and TOS allows a full assessment of oxidative stress.[32] In this study, TAC and TOS levels were examined as oxidative stress parameters. TAC levels were similar in the control and hemodialysis groups and were not statistically significant. We were expecting the decrease of TAC levels but decreases were not determined. It could be due to uric acid concentration as reported by Jackson et al.[30] In kidney diseases, uric acid level increases.[33] In some environments, urate is an effective antioxidant against ozone-based radicals. In addition, it is not a biologically important radical scavenger and it is unlikely that an increase in urate concentrations will provide an adequate antioxidant defense in the current deficiency in other antioxidant systems. In addition, urate-derived radicals can cause tissue damage in ascorbate deficiency.[30,34] Again, in this study, the level of TOS was found to be statistically significantly higher in the HD group compared to the controls. Therefore, hemodialysis patients are at risk for cardiovascular diseases due to high oxidative stress.[35] The importance of antioxidants should not be forgotten to reduce oxidative stress.

It is necessary to understand what causes the consistent activation of inflammation. Inflammation is a physiological course that is beneficial in the short period but causes several complications when persistently activated. Indeed, the inflammatory process is a physiological protective mechanism for protecting the host against infections, tissue repair, adaptation to stress and restoration of the homeostatic state.[36] A controlled inflammatory response is beneficial by eliminating the stimuli that damage the host and initiating the healing procedure in the tissue; it can also be harmful if it becomes irregular. It should be noted that permanent inflammation is a common phenomenon in many chronic diseases due to aging and the burden of life.

It is noteworthy that permanent inflammation is a widespread event in many chronic diseases due to aging and the burden of lifestyle. Permanent inflammation is thought to contribute to numerous complications such as atherosclerosis, osteoporosis, fragility, diabetes, cancer and depression, and the chronic inflammatory condition accompanies chronic kidney disease.[37]

The inflammatory response is particularly regulated by cytokines such as TNF-α and IL-6. Both regulate the production of acute phase proteins that have an effect on lipid and carbohydrate metabolism and are associated with an increased risk of coronary artery disease in individuals with normal renal function.[38,39] Proinflammatory cytokines regulate vascular adhesion. TNF-α specifically stimulates the production of the soluble cell adhesion molecule by endothelial cells.[40] The increased plasma level of the fusible cell adhesion molecule has been reported in patients with chronic renal failure.[41]

Circulating levels of proinflammatory cytokines such as IL-6 and IL-18 are high in end stage kidney patients. Increased proinflammatory cytokines play a very important role in chronic inflammation. It is associated with cardiovascular incidents and bad results in dialysis patients.[42-45] Among the inflammatory cytokines, high IL-18 levels have been shown to be higher in the rate of hospitalization of dialysis patients, possibly due to cardiovascular mechanisms.[42,46] The results from experimental and clinical studies display that IL-18 is firmly related to atherosclerotic plaque progression and fragility.[47] In addition, daily IL-18 administration has been reported to cause myocardial dysfunction in healthy mice.[48] Therefore, it is proposed that IL-18 probably causes left ventricular dysfunction by aggravating coronary atherosclerosis or by directly acting on cardiomyocytes to stimulate myocardial dysfunction.[48,49] Increased circulating IL-18 levels have proven to be a strong and independent prediction for cardiovascular death in patients with coronary artery disease (CAD).[50] However, it is still unclear whether a higher IL-18 level is associated with mortality and whether IL-18 is useful for early risk classification in dialysis patients.[51]

Inflammation and oxidative stress increase in uremic patients. Oberg et al.[35], found that oxidative stress and inflammation markers increased in patients with chronic kidney failure compared to healthy people. Increased oxidative stress and inflammation in uremia may be responsible for the increase in the rate of cardiovascular mortality and morbidity in uremic patients. There are also studies indicating that increased indicators of inflammation (especially hs-CRP and IL-6) in uremic patients are a strong and independent predictor for developing cardiovascular mortality and morbidity.[52] Borazan et al.[53] found that TNF-α, IL-6 and CRP levels, which they examined in peritoneal dialysis and HD patients, were statistically significantly higher than the control group level.

In this study, TNF-α, IL-6, IL-18 and CRP levels were evaluated as inflammatory markers. TNF-α, IL-6, IL-18 and CRP levels of HD group were statistically significantly higher at the level, compared to the control group. It is clearly seen that inflammation increases in HD patients. Therefore, inflammation-reducing strategies are important. Physical exercise, balanced diet and non-smoking healthy lifestyle should be recommended in terms of reducing inflammation. Additionally, many medications widely used in the treatment of patients with chronic kidney failure and other common pathological conditions have shown a possible positive effect on inflammation. These drugs include statin, angiotensin-converting enzyme inhibitors and vitamin D. In addition, new anti-inflammatory drugs have been produced that target pro-inflammatory cytokines. Available data on their effectiveness in chronic kidney patients are scarce and often uncertain. Its benefits are obtained from the results of general population or chronic patients. Specific anti-inflammatory drugs such as thalidomide, pentoxifylline and tocilizumab and canakinumab can be counted in this group. These drugs have

proven to be valuable in other persistent inflammatory diseases.[37]

Conclusion

As a result; in this study, cystatin C levels, oxidative stress parameters and levels of inflammatory biomarkers were investigated in HD patients. Cystatin C, TOS, TNF-α, IL-6, IL-18 and CRP levels were statistically higher than controls. Consequently, in hemodialysis patients, oxidative stress and inflammation increased. Due to the detected oxidative stress and inflammation, new studies on HD patients and new strategies to reduce inflammation with oxidative stress are needed.

Conflict of Interest

The authors declare no conflict of interest.

Compliance with Ethical Statement

Ethics committee and approval of the study was obtained from Van Yuzuncu Yıl University Non-Interventional Clinical Research Ethics Committee (Ethics Committee Approval No: 2019/09-05).

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Author Contributions

Study Idea/Hypothesis and Desing: HM; Data Collection: MD, NO; Analysis: HM, LM, MD; Manuscript writing: HM, NM; Critical Review: HM, NM; Publishing Process: HM, LM.

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