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Original Investigation

The Investigation of Relationship Between Secondary Hyperparathyroidism and Oxidative Stress in Patients with Chronic Kidney Disease

Kronik Böbrek Yetmezlikli Hastalarda Sekonder Hiperparatrodizm ve Oksidatif Stress Arasındaki İlişkinin Araştırılması

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ABSTRACT

OBJECTIVES: Our aim was to investigate the changes of the oxidant and antioxidant systems in chronic kidney disease patients who had increased parathyroid hormone (PTH) levels in the present study.

PATIENTS and METHODS: A total of fifty hemodialysis patients were divided into two equal groups based on the PTH levels; > 300 pg/mL (Group 1) and < 300 pg/mL (Group 2). A total 20 healthy subjects were included in the study as the control group (Group 3). The measurement of malondialdehyde (MDA), advanced protein oxidation products level (AOPP), ascorbic acid (AA) levels, and activities of myeloperoxidase (MPO) and catalase (CAT) were performed by colorimetric methods. PTH measurement was also performed by chemiluminescent method.

RESULTS: As compared with control group, MDA level was significantly higher in Groups 1 and 2 ($p<0.05$), and the AOPP level was higher in Group 2 ($p<0.05$) only. The MPO activity was significantly lower in Group 1 as compared to the control group ($p<0.05$). The CAT activity and AA levels were significantly lower in Groups 1 and 2 than in the control group ($p<0.05$).

CONCLUSIONS: This study has shown that patients with chronic kidney disease are characterized by increased oxidative stress and decreased antioxidant defense systems. However, increased PTH levels did not have an additional effect on the oxidant and antioxidant mechanisms in these patients.

KEY WORDS: Antioxidant, Heamodialysis, Hyperparathyroidism, Renal failure, Oxidative stress

ÖZ

AMAÇ: Bu çalışmada, paratroid hormon (PTH) seviyesi yüksek kronik böbrek yetmezlikli hastalarda, oksidan ve antioksidan sistemlerdeki değişikliklerin araştırılması amaçlanmıştır.

GEREÇ ve YÖNTEM: Toplam 50 hemodiyaliz hastası PTH düzeyleri esas alınarak iki eşit gruba bölündü; PTH düzeyi > 300 pg/mL (Grup 1) ve < 300 pg/mL (Grup 2). Yirmi sağlıklı kişi kontrol grubu (Grup 3) olarak çalışmaya katılmıştır. Malondialdehit (MDA), ilerlemiş protein oksidasyon ürünü (AOPP), askorbik asit (AA) düzeyleri ve myeloperoksidaz (MPO) ve katalaz (KAT) aktivitesi ölçümleri kolorimetrik yöntemle gerçekleştirilmiştir. PTH ölçümü ise kemiluminens yöntemle gerçekleştirilmiştir.

BULGULAR: Kontrol grubuyla karşılaştırıldığında, MDA düzeyi grup 1 ve 2'de, AOPP düzeyi ise sadece grup 2'de anlamlı artış göstermiştir ($p<0.05$). MPO aktivitesi ise grup 1'de kontrol grubuna göre anlamlı azalma göstermiştir ($p<0,05$). KAT aktivitesi ve AA düzeyleri, kontrol grubuna göre, hem grup 1 hem de grup 2'de anlamlı olarak azalmıştır ($p<0,05$).

SONUÇ: Bu çalışma, kronik böbrek yetmezlikli hastaların artmış oksidatif stres ve azalmış antioksidan savunma sistemiyle karakterize olduğunu göstermiştir. Bununla birlikte, PTH yüksekliğinin bu hastalarda oksidan ve antioksidan mekanizma üzerinde herhangi bir etkiye sahip olmadığını göstermiştir.

ANAHTAR SÖZCÜKLER: Antioksidan, Böbrek yetmezliği, Hemodiyaliz, Hiperparatroidizm, Oksidatif stres

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INTRODUCTION

Chronic kidney disease (CKD) is a chronic and progressive disease characterized by renal dysfunction due to the decrease of the glomerular filtration rate (GFR) (1). Increased oxidative stress is now considered one of the major risk factor in CKD patients, particularly those treated by hemodialysis (HD). Oxidative stress is defined as a loss of the normal balance between reactive oxygen species (ROS) production and the antioxidant system (2). Indeed, in addition to an excess generation of ROS, uraemic patients have decreased antioxidant capacity, which causes oxidative damage to cells (3).

The oxidative stress in patients with HD is traditionally attributed to the recurrent activation of polymorphonucleate neutrophils and monocytes. These are activated by the concurrent effect of blood passing through the dialysis circuits, the components being activated following contact with membranes that are of poor compatibility, as well as the possible passage of dialysate endotoxins, consequent to back-filtration (4,5). The action of ROS on the biological tissues leads to a harmful oxidating effect on all their biochemical components: lipids, proteins, carbohydrates, and nucleic acids. Following the peroxidation of lipids, they become ruptured and release aldehydes: malonildialdehyde (MDA) and ketones. Currently, MDA assay is one of the most widely used methods for the evaluation of lipoperoxidation (5,6). Oxidation of tyrosine residues leads to the formation of dityrosine residues, with aggregation, cross linking, or fragmentation of the proteins themselves (7). New compounds and modified structures including advanced oxidation protein products (AOPP) are formed (8). These proteins are characterized by their high molecular weight, which reduces both renal and dialytic clearance. Phagocytic cells contain the haem enzyme myeloperoxidase (MPO) which catalyses the reaction of chloride ion with hydrogen peroxide (H2O2) to generate large amounts of hypochlorous acid (HOCl), a powerful oxidizing and microbicidal agent. The deleterious effect of HOCl is directed not only at micro-organisms but also at bystander host cells during inflammation (9-11).

Secondary hyperparathyroidism (SHPT) is one of the most important complications in chronic HD patients. It is associated with mortality in patients undergoing dialysis treatment. It is reported that over-secretion of parathyroid hormone (PTH) because of SHPT influences not only bone and mineral metabolism, but also various organs such as the central nervous system, cardiovascular system, haematopoietic system, immune system and lipid metabolism (12,13). There are many studies that have investigated the association between CKD and oxidative stress (3,4,7). To our knowledge, our study is the first to investigate the effects of hyperparathyroidism on oxidant and antioxidant systems in patients with CKD treated by HD.

MATERIAL and METHODS

A total of fifty CKD patients who underwent hemodialysis treatment were included in the present study. These patients were divided into two equal groups based on the PTH levels: one group of CKD patients had PTH > 300 pg/mL (Group 1), and other < 300 pg/mL (Group 2). Group 1 consisted of 17 males and 8 females, aged 22-72 yr (average, 46.60±2.97 yr), and Group 2 consisted of 13 males and 12 females, aged 25-74 yr (average, 48.08±3.21 yr). The Control Group (Group 3) consisted of 20 subjects, 14 males and 6 females, aged 22-65 yr (average, 43.65±3.29 yr). The control subjects had normal PTH levels and renal function tests. Previous renal disease and the use of any drug were taken as exclusion criteria in the control subjects.

Patients with CKD were dialysed three times a week using polysulphon membrane, each session lasting 4 hours with bicarbonate dialysate. Dialysis performance was satisfactory. Dialytic adequacy (Kt/V) was greater than 1.3. Blood samples were collected before the second dialysis. A total of 10 mL of venous blood was taken from all subjects. All blood samples were drawn into test tubes that did not include anticoagulant substance for measuring biochemical parameters. The tubes were centrifuged for 10 min at 3000 rpm and all samples were stored at –70 °C until they were analysed.

The serum levels of thiobarbituric acid-reacting substances (TBARS), an end product of lipid peroxidation, were measured fluorometrically at wavelengths of 525 nm for excitation and 547 nm for emission (14). Serum MDA concentration was expressed as nmol/mL.

Determination of AOPP was based on a spectrophotometric assay according to Witko-Sarsat et al., 1996 (7). AOPP levels were expressed in μmol of chloramine-T equivalents per litre of serum (μmol/L).

Serum MPO activity was determined by the method of Bradley et al, 1982, and was based on kinetic measurement of the formation rate of the yellowish-orange product of the oxidation of o-dianisidine with MPO in the presence of hydrogen peroxide (H_2O_2) at 460 nm (15). One unit of MPO was defined as that degrading 1 μmol of H_2O_2 per minute at 25°C. A molar extinction coefficient of 1.3*104 M-1 cm-1 of oxidized 0-dianisidine was used for the calculation. MPO activity was expressed in units per liter of plasma (U/L).

The serum catalase (CAT) activity was determined by method of Goth et al, 1991, in which a homogenate was incubated with H_2O_2 substrate and the enzyme reactions were stopped by the addition of ammonium molybdate (16). Serum CAT activity was expressed as U/L.

Serum ascorbic acid (AA) levels were estimated calorimetrically using a DTCS reagent prepared by mixing dinitrophenyl hydrazine, thiourea, and copper sulfate in a 1:1:20 ratio, according to the method developed by Teitz, 1999 (17). Serum AA concentration was expressed as mg/dL.

The serum PTH levels were measured using the Immulite kit, which is a solid-phase two-site chemiluminescent immunometric assay and biochemical measurements were performed using a Hitachi PP Modular Automatic analyser with Roche original reagents. Very low density lipo protein (VLDL)-cholesterol level was calculated with the Frieldweld formula.

Statistical Analysis

The results are expressed as mean (X) ± standard error (SE). The Kolmogorov-Smirnov normality test was applied for all variables. All variables showed normal distribution. The Pearson correlation analysis was then used to determine the relationships among the variables and one-way ANOVA was employed to compare group means. In addition to ANOVA, the Tukey test was used as multiple comparison tests.

RESULTS

The clinical characteristics of the Groups are given in Table I. The body mass index was significantly lower in Groups 1 and 2 than in Group 3 ($p<0.05$).

Biochemical characteristics of the Groups are presented in Table II. Serum urea and PTH levels were significantly increased in Groups 1 and 2 when compared to the control group ($p<0.01$), and these parameters were also significantly increased in group 1 compared to group 2 ($p<0.05$). Significantly increased serum creatinine and phosphorus and decreased albumin and total cholesterol levels were found in Groups 1 and 2 when compared with the control group ($p<0.05$). LDL cholesterol level was found to be decreased in Group 1 compared with the control group ($p<0.05$).

Descriptive statistics regarding the oxidant and antioxidant parameters of all Groups are presented in Table III. Increased MDA levels were found in the Groups 1 and 2 as compared with the control group ($p<0.05$). The AOPP level was significantly higher than the Control Group only in group 2 ($p<0.05$). MPO activity was significantly decreased in Group 1 compared to the control group ($p<0.05$). CAT activity was also significantly decreased in Group 1 compared with Groups 2 and 3 ($p<0.05$). A significantly decreased AA level was found in Groups 1 and 2 compared to the control group ($p<0.05$).

Table IV depicts the correlation between the parameters in Group 1. In Group 1, there was a significant positive correlation between the CAT and phosphorus ($p<0.05$, r=0,484), uric acid ($p<0.05$, r=0,487), and AA ($p<0.05$, r=0.451), MPO and PTH ($p<0.01$, r=0,624), creatinine ($p<0.05$, r=0.469) and phosphorus ($p<0.05$, r=0,454), AOPP and PTH ($p<0.05$, r=0.516), uric acid and phosphorus ($p<0.01$, r=0,588), PTH and creatinine ($p<0.05$, r=0,575), and urea and phosphorus ($p<0.05$, r=0,471).

The regression analysis results for the oxidant and antioxidant parameters are presented in Table V.

DISCUSSION

Chronic renal failure is associated with increased formation of ROS and impaired antioxidant defense.

Table I. The clinical characteristics of the study groups.

Clinical characteristics	Group 1 X±SE	Group 2 X±SE	Group 3 X±SE
Man (n)	17	13	14
Women (n)	8	12	6
Age (year)	46.60±2.97	48.08±3.21	43.65±3.29
BMI (kg/m^2)	22.12±0.62 [a*]	22.43±0.56 [a*]	26.83±1.10
Dialysis time (month)	35.44±5.84	38.64±5.05	

[*]p<0.05; [a]compared to group 3, BMI: body mass index.

Table II. The comparison of biochemical parameters between all groups.

Parameters	Group 1 (n=25) X±SE	Group 2 (n=25) X±SE	Group 3 (n=20) X±SE
Glucose (mg/dL)	109.7±13.1	97.56±7.11	86.95±3.12
Urea (mg/dL)	155.28±6.77 ^{a*,b**}	129.52±9.84 ^{b**}	33.35±1.76
Creatinine (mg/dL)	6.75±0.88 ^{b**}	4.73±0.52 ^{b**}	0.87±0.35
Uric acid (mg/dL)	5.15±0.56	6.05±0.41	5.55±0.28
Albumin (g/dL)	3.94±0.76 ^{b*}	3.92±0.86 ^{b*}	4.51±0.06
Triglyceride (mg/dL)	129.0±9.80	161.1±11.0	173.8±16.6
Total cholesterol (mg/dL)	139.36±5.87 ^{b*}	161.96±7.32 ^{b*}	190.45±9.50
HDL-cholesterol (mg/dL)	40.0±2.0	39.72±3.08	45.90±1.90
LDL-cholesterol (mg/dL)	72.32±4.87 ^{b*}	88.28±6.35	105.35±8.12
VLDL-cholesterol (mg/dL)	25.84±1.95	32.16±2.18	39.20±4.78
Calcium (mg/dL)	9.32±0.23	9.42±0.28	9.61±0.07
Phosphorus (mg/dL)	5.09±0.60 ^{b*}	4.60±0.40 ^{b*}	3.24±0.14
PTH (pg/mL)	666.8±58.4 $^{a*, b**}$	195.0±11.5 ^{b*}	38.03±3.94

$^{*}p<0.05$; $^{**}p<0.01$; ^{a}compared to group 2; ^{b}compared to group 3, HDL: high dansity lipoprotein, LDL: low dansity lipoprotein, VLDL: very low-dansity lipoprotein, PTH: parathormon.

Table III. The comparison of oxidant and antioxidant parameters in all groups.

Parameters	Group 1 X±SE	Group 2 X±SE	Group 3 X±SE
MDA (nmoL/mL)	3.46 ± 0.16 ^{b*}	3.59 ± 0.19 ^{b*}	2.54 ± 0.11
AOPP (μmoL/L)	187.37 ± 16.37	213.03 ± 20.25 ^{b*}	133.77 ± 19.74
MPO (U/L)	53.35 ± 5.20 ^{b*}	71.70 ± 11.59	126.14 ± 13.10
CAT (kU/L)	34.00 ± 4.20 $a^{*,b*}$	56.75 ± 9.02	66.54 ± 9.00
AA (mg/dL)	0.81 ± 0.10 ^{b*}	0.69 ± 0.11 ^{b*}	1.28 ± 0.14

$^{*}p<0.05$; ^{a}compared to group 2; ^{b}compared to group 3, MDA: malondialdehyde, AOPP: advanced protein oxidation products, MPO: myeloperoxidase, CAT: catalase, AA: ascorbic acid.

Oxidative stress is an important risk factor for the development and progression of several complications in HD patients. HD may cause increased ROS production and diminished antioxidant reserve by itself (18,19). In the present study, increased MDA levels were determined in each group of HD patients. However, increased PTH levels did not show any additional effect on MDA levels. There are various reports about effect of PTH on vascular endothelial cell function. Recently, Rashid et al have reported that elevated PTH levels might stimulate the production of reactive oxygen species and found that PTH stimulated up-regulation of mRNA of the receptor for advanced glycation end products in cultured endothelial cells (20). PTH has also been linked to an increase in the vascular smooth muscle cell synthesis of 20-hydroxyeicosatetranoic acid, which is a constrictive agent (21). The cause of oxidative stress on HD patients might be via different mechanism. Roman et al reported the main cause of increased oxidative stress as HD treatment (22). However, the main cause of oxidative stress is chronic renal failure according to Floccari et al (5). The relationship between the MDA production and HD treatment is very important. The three main mechanisms that may cause the oxidative stress in HD patients are uraemia, the dialysis membrane, and bacterial contamination from the dialysate (5). Ozden et al have reported that circulating MDA levels seem to be higher at the end of the HD session than before dialysis, and

Table IV. The correlation between the measurement parameters in group 1.

	MDA	CAT	MPO	AA	AOPP	UA
CAT	-,277					
MPO	,079	,287				
AA	-,206	**,451***	,256			
AOPP	-,201	-,048	,213	,196		
UA	-,176	**,487***	,244	,207	-,027	
PTH	,118	-,043	**,624****	,077	**,516***	-,098

** p< 0.01 ,*p< 0.05, MDA: malondialdehyde, CAT: catalase, MPO: myeloperoxidase, AA: ascorbic acid, AOPP: advanced protein oxidation products, UA: uric acid, PTH: parathyroid hormone.

Table V. The regression analysis results for oxidant and antioxidant parameters (the independent variable is PTH)

	Group 1			Group 2		
Parameters	b	t	R^2	b	t	R^2
MDA	0.001	0.569	0.014	-0.002	-0.578	0.014
AOPP	0.145	2.759*	0.266	-0.195	-0.542	0.013
MPO	0.056	3.832**	0.39	-0.059	-0.129	0.001
CAT	-0.003	-0.207	0.002	-0.066	-0.403	0.007
AA	0.001	0.373	0.006	0.002	0.948	0.038
UA	-0.001	-0.471	0.01	0.016	2.344*	0.193

** p< 0.01 ,*p< 0.05, MDA: malondialdehyde, CAT: catalase, MPO: myeloperoxidase, AA: ascorbic acid, AOPP: advanced protein oxidation products, UA: uric acid, PTH: parathyroid hormone.

this suggests that a single session can play an active role, triggering lipoperoxidation (23). Bio-incompatibility of the dialysis membrane might result in activated neutrophils which stimulate the release of free radicals.

Proteins are among the most vulnerable targets of ROS. Oxidation of tyrosine residues leads to the formation of dityrosine residues, with aggregation, cross linking, or fragmentation of proteins themselves. AOPP is the generic name coined to indicate the grouping of proteins modified by oxidative stress (7,24). In the present study, an increased AOPP level was found in Group 2 as compared to controls. However, no other significant difference was observed between the other Groups. Witko-Sarsat et al, and Wratten et al reported that circulating levels of AOPP are high in patients on hemodialysis (7,25). However, Witko-Sarsat et al reported an inverse relation between the creatinine clearance and AOPP levels in pre-dialysis patients (26). Protein oxidation may thus be both the cause and the result of oxidative stress in uremic patients, causing a true and proper self-perpetuating vicious circle. In the present study, patients with CKD were characterized by decreased serum albumin levels. It might be because of both increased catabolism and insufficient nutrition of these patients. Thus, albumin was the major plasma protein target of oxidative stress in uraemia and its oxidation contributes to the development of cardiovascular risk (27). Moreover, biochemical analyses have shown that plasma AOPP were cross-linked oxidized proteins, mostly albumin (28).

Myeloperoxidase, abundantly stored in primary granulocytes of polymorphonuclear leukocytes and released from degranulated neutrophils, has been considered as an important pathophysiological factor in oxidative stress during the HD process and may serve as a reliable indicator of oxidative stress during single HD sessions. Confusingly, decreased MPO activity was found in Group 1 as compared to the Control Group. It is important to stress that the increase in basal neutrophil MPO activity is not related to soluble plasma MPO but is due to intracellular MPO activity. Likewise, Witko-Sarsat et al reported an increase in basal oxidase and MPO activities in isolated neutrophils from HD patients, in the

absence of plasma components (29). The kinetic changes of MPO at different access sites during a single HD session using a biocompatible membranes and ultrapure dialysate reveal the differences in MPO production between arterial and venous sites (30). A recent study results reported by Krieter et al showed significantly changed MPO activity during the dialysis session, and there were also significant differences between arterial and venous values as regarding MPO activity (31). The blood samples were obtained before the HD session in the present study. Therefore, the decreased MPO activity in Group 1 might be associated with the time the sample was obtained. A prominent difference in MPO production between two different biocompatible dialysis membranes in previous studies may imply that the bioincompatibility of the membranes also seems to play an important role in the increased production of oxidative stress. Another possible reason for the difference regarding the MPO results might be the role of heparin in the MPO release during the HD session. Léculier C et al have reported that high molecular weight (HMW) and low molecular weight (LMW) heparins affected superoxide ion production and degranulation by polymorphonuclear leukocytes (PMNL) isolated from either chronic hemodialyzed patients or healthy controls, MPO released by PMNL was also effected by HMW and LMW-heparins as dose-dependent in either chronic hemodialyzed patients or healthy controls (32). In addition, Leitienne et al reported LMW heparin used at regular high dose during a 4-h dialysis session induces a less granulocytopenic effect as compared with the usual high dose of unfractioned heparin (33). Taking all our findings into consideration, not only the interaction with the dialyser but also other factors mentioned above should be considered as contributing to MPO release in HD patients.

A weakening of antioxidant defenses caused by HD may be characterized by leakage and consumption of hydrophilic antioxidants during dialysis; consumption of liposoluble/lipoprotein-associated antioxidants; changes in the lipid composition of biological fluids and cell membranes and/or a deficit in cofactors and damage to antioxidant enzymes (34). A decrease in antioxidant CAT activity and AA levels in CKD patients was observed in the present study, which means that these patients may be more susceptible to oxidative damage by free radicals. The other cause of decrease on antioxidant parameters might depend on sample handling time, technique, membrane biocompatibility and dialyzate. González-Diezm et al recently reported different activities of superoxide dismutase and glutathione peroxidase after the dialysis session as compared to before (35). However, the data of HD patients regarding antioxidant enzyme activities are controversial. The activities of these enzymes were variably reported to be decreased, increased or unchanged in HD patients (36,37,38,39). Yilmaz et al have reported similar vitamin C levels between the pre-dialysis group and controls but the levels were significantly reduced in the post-dialysis group when compared with the pre-dialysis and control samples (40). The reduced detoxifying capacity of the main enzymatic anti-oxidant systems deprives the uremic subject of the main tool of defense against aggression from these compounds, thus contributing to a breakdown in the delicate balance between oxidating and anti-oxidating systems.

In conclusion, as similar to previous studies, the results of this study indicate that hemodialysis patients are characterized by increased oxidative stress and decreased antioxidant activity. Increased PTH levels also did not cause any additional effect on oxidative stress. However, increased PTH levels caused a decrease of catalase activity. We think that our study needs to be supported by further studies.

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