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Evaluation of the Relationship Between Advanced Oxidation end Products and Inflammatory Markers in Maintenance Hemodialysis Patients

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Abstract

Introduction: Increased oxidative stress and blunted anti-oxidant mechanisms are important problems in hemodialysis (HD) patients. Reactive oxygen species (ROS) act directly on proteins, leading to the formation of oxidized amino acids. Advanced oxidation protein products (AOPP) are among these substances. Many oxidant substances increase the level of AOPP. Iron is an element with strong oxidant capacity, especially when used intravenously. It is thought that iron treatment further increases the oxidative stress in HD patients. We aimed to investigate the relationship between AOPP and inflammatory status in HD patients.

Materials and Methods: Patients who were on maintenance HD program without additional co-morbidities and no history of use of intravenous iron within the last two weeks were recruited in the study. The blood samples taken just before the dialysis session were analyzed for AOPP, serum iron, total iron binding capacity (TIBC), ferritin, C-reactive protein (CRP), ß2-microglobulin, fibrinogen, interleukin (IL)-1, IL-6 and tumor necrosis factor-α levels besides routine biochemical measurements and complete blood count.

Results: The number of patients included in the study was 102 (n: 53 female, %52.0) and the mean age was 47.6±13.9 years. The mean transferrin saturation was 25.4%. AOPP levels, iron use in patients was higher compared to patients who do not use (respectively 2.58±0.19 mmol/l and 2.50 ±0.16mmol/l, p = 0.046). We did not detect statistically significant correlation of AOPP levels with iron parameters and other inflammatory markers.

Conclusion: The present study showed that intravenous iron therapy does not increase oxidative stress. Although serum AOPP level was higher in patients on intravenous iron treatment, it was not correlated with iron indices and inflammatory markers. So, intravenous iron may exert its oxidant effect free from serum iron indices.

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Introduction

Reactive oxygen species (ROS) and antioxidant mechanisms are in balance in human body under normal physiological conditions. Overproduction of ROS or attenuated antioxidant defense mechanisms causes oxidative stress by way of structural and functional modifications in biomolecules. Increased oxidative stress and attenuated antioxidant mechanisms are important problems in hemodialysis patients.[1] Bio-incompatible hemodialysis membranes, contaminated dialysis solutions and other similar causes stimulate release of proinflammatory cytokines like interleukin (IL)-1, IL-6 and tumor necrosis factor-alpha (TNF-α).[2] Polymorphonuclear leukocytes which are activated by cytokines release ROS that in turn activates nuclear factor kappa-B (NF-κB), the transcription factor of cytokines, beginning a vicious circle between cytokines and ROS.[3]

Reactive oxygen species causes oxidation of amino acids directly. ROS also causes formation of advanced glycation end products and advanced lipoxidation end products indirectly through carbonyl compounds formed by auto oxidation of carbohydrates and lipids.[4] ROS causes aggregation and fragmentation in protein structures by way of directly oxidizing tyrosine residues with the resultant formation of dityrosine. The resultant products are called '*advanced oxidation protein products*' (AOPP).[5]

Anemia of chronic renal failure requires treatment with iron and/or erythropoietin. Iron is an element with strong oxidant capacity. AOPP levels increase in hemodialysis (HD) patients especially in conjunction with inflammation and iron treatment.[6,7] Iron in the treatment dose causes increase in the serum free iron which in turn converts into hydroxyl radical by reacting with hydrogen peroxide through phenton reaction.[8] There is chronic inflammation in most of the HD patients. This may be a component of malnutrition-inflammation-atherosclerosis (MIA) syndrome.[9] All three components of this syndrome cause increased morbidity and mortality in end stage renal disease (ESRD). These HD patients have elevated levels of proinflammatory cytokines and high sensitive C-reactive protein (hsCRP).

Advanced oxidation protein products, which was first described by Witko-Sarsat et al.[5] in the plasma of uremic patients as highly oxidized protein products, is known as a reliable marker of oxidative stress in chronic renal failure (CRF). AOPP, the structure of which continues to be studied, has a high molecular weight which decreases its renal clearance. AOPP has been shown to be formed of albumin aggregates with disulfide bonds and/or tyrosine cross bonds. It is different from pure albumin by chromatographic and electrophoretic techniques. The formation of AOPP is irreversible: they cannot be easily hydrolysed by proteases or reduced by antioxidants and are mostly eliminated by the liver and spleen.

Detection of AOPP related parameters may affect the approach to prevent oxidative stress in maintenance HD patients. We aimed in this study to examine the relationship between AOPP levels, iron indices and inflammatory markers such as CRP, ß2-microglobulin, fibrinogen, IL-1, IL-6, TNF-α in chronic HD patients.

Materials and Methods

Patients

The number of patients included in the study was 102 (n: 53 female, %52.0) and the mean age was 47.6±13.9 years. Patients having HD treatment thrice a week for at least three months were included in the study. Synthetic dialyzers were used for HD sessions. An informed consent form was received from each patient.

Exclusion criteria; Patients with HD schedule other than thrice a week, dialysis duration less than three months, those with acute kidney, active gastrointestinal or genitourinary bleeding, clinically active infection, hemoglobin level less than 8 g/dl, a change in vascular access during the study, patients who used steroids or any other immunosuppressive drug, those with acute coronary syndrome, advanced heart failure, hepatitis B or C, or any other disease that may cause inflammation and/or oxidative stress, pregnant women and patients who did not give informed consent were excluded from the study.

To eliminate the potential oxidative effects of iron, only patients who did not have oral or intravenous iron therapy within the last two weeks were included.

Demographic data of the patients such as age, sex, weight, height, body mass index (BMI) and primary renal disease were recorded.

Laboratory Analysis

Blood samples (15 ml) were obtained from patients before the midweek HD session for measurement of serum urea, creatinine, cholesterol, triglyceride, calcium, phosphorus, parathyroid hormone, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), hemoglobin, hematocrit, leukocyte, iron, total iron binding capacity (TIBC), ferritin, CRP, ß2-microglobulin, fibrinogen, IL-1, IL-6, TNF-α, ROS and AOPP levels. Albumin, AST, ALT, cholesterol, triglyceride, iron, ferritin, TIBC and CRP levels were studied by COBAS MIRA auto analyzer with methods proposed by the manufacturer. Phosphorus and calcium levels were measured by ETACHEM 250 analyzer. Parathyroid hormone level measurement was performed with immunoradiometric assay. Plasma AOPP level was measured as double copies with the method adopted to COBAS MIRA and with the original method described by Witko-Salsat et al.[5] Fibrinogen level was determined by clot-ray assay using Diagnostica STAGO ST4 machine. TNF-α, IL-1 (Genzyme Diaynostics) and IL -6 (Endogen) levels were studied by enzyme-linked immunosorbent assay (ELISA) method. ß2-microglobulin levels were measured by nephelometric method using Immage Instrument (Beckman Coulter).

Statistical Analysis

Statistical analysis was performed by SPSS (Statistical Package for Social Sciences) for *Windows 22.0* (standard version). Numerical variables that exhibited normal distribution were given as mean ± standard deviation (SD). Comparison of the groups with/ without intravenous iron therapy were performed with independent sample *t* test. The Spearman rank correlation was used to evaluate correlations. Multivariate analysis was performed with linear regression (stepwise method). Statistical significance was defined by $p<0.05$.

Results

A total of 102 patients (53 female, 49 male) were used in the study. The mean age of the patients was 47.6±13.9 years. The most common reason for ESRD was diabetic nephropathy (n=22, 21.6%). The demographic data and laboratory results of the patients are presented in Table 1.

The mean transferring saturation of the patients was 25.4%, 30 patients did not receive intravenous iron (iron sucrose) treatment. 72 patients were using intravenous iron except for the last two weeks. AOPP and fibrinogen levels were higher in patients on intravenous iron (AOPP: 2.58 ±0.19 mmol/L vs. 2.50±0.16 mmol/l, p=0.046; fibrinogen: 284±6 mg/dl vs. 280±13 mg/dl, p=0.045). The mean IL-1 level was lower in patients on iron therapy (11.80±0.58 pmol/L vs. 11.54±0.53 pmol/L, p=0.029). CRP, IL-6, ROS and TNF-α levels were similar in patients receiving or not receiving iron (Table 2).

Advanced oxidation protein products level was found to be correlated positively with ROS (r= 0.297, p=0.003) and negatively with BMI (r=-0.237, p=0.017), calcium (r=-0.262, p=0.008) and IL-1 (r=-0.205, p=0.039) with univariate analysis. There was no significant correlation between AOPP levels and demographic parameters (age, gender, presence of diabetes mellitus, use of intravenous iron therapy), biochemical parameters (urea, creatinine, albumin, total cholesterol, triglyceride, phosphorus, parathyroid hormone, AST, ALT), iron indices (hemoglobin, hematocrit, iron, TIBC, ferritin) and inflammatory markers (fibrinogen, IL-1, IL-6, TNF-α and CRP).

On multivariate analysis. AOPP level was found to be related only with BMI and ROS levels (respectively; B=0.088, beta=0.030, p= 0.004, B=0.008, beta=-0.003, p=0.027).

Discussion

Increased oxidant stress and attenuated antioxidant defense mechanisms are important problems in HD patients.[1] Uremic toxic metabolites, the dialysis procedure itself, loss of trace elements during HD and thermal injury increase oxidative stress. Inhibition of antioxidant enzymes by uremic toxins, decreased function of renal antioxidant enzymes and deficiencies of copper, zinc and selenium weakens the antioxidant defense.[10-12]

Colombo et al.[13] evaluated the association between AOPP levels, C-reactive protein concentration

Table 1. Results of demographic characteristics, primary kidney diseases, iron related parameters, inflammatory biomarkers of the patients of the study group.

		Mean ± Standard Deviation
Demographic characteristics		
Age (year)		47.6 ±13.9
Weight (kg)		66.8 ±15.3
Height (cm)		162.7 ±9.2
Primary kidney disease	Diabetic nephropathy	22 (21.6%)
	Chronic glomerulonephritis	15 (14.9%)
	Primary nephrosclerosis	12 (11.9%)
	Chronic pyelonephritis	9 (8.9%)
	ADPCKD	5 (5.0%)
	Secondary amyloidosis	3 (3.0%)
	Other or unknown	36 (35.6%)
Albumin (g/dl)		3.51±0.23
Calcium (mg/dl)		9.57 ±0.16
Phosphorus (mg/dl)		5.52 ±0.14
Parathormon (pg/dl)		346 ±33
AST (U/I)		26 ±5
ALT (U/I)		26 ±3
Iron related parameters		
Hemoglobin (g/dl)		10.4 ±1.4
Iron (mg/dl)		89 ±5
TIBC (mg/dl)		354 ±12
Ferritin (ug/I)		2157 ±14
Inflammatory parameters		
AOPP (mmol/I)		2.56 ±0.19
Fibrinogen (mg/dl)		283 ±9
IL-1 (pmol/L)		11.6 ±0.5
IL-6 (pmol/L)		13.3 ±0.9
TNF- α (pmol/L)		12.9 ±1.0
CRP (mg/dl)		6.86 ±1.35
ROS (µmol/L)		18.8 ±0.6

ADPCKD: Autosomal dominant polycystic kidney disease. AST: aspartate aminotransferase. ALT: alanin aminotransferase. TIBC: Total iron binding capacity. AOPP: Advanced oxidation protein products. IL: interleukin. TNF- α: tumor necrosis factor-alpha. CRP: C-reactive protein. ROS: Reactive oxygen species.

Table 2. In hemodialysis patients, intravenous iron use effects on oxidative stress and inflammatory markers.

	Intravenous iron intake		p
	No n=30	Yes n=72	
AOPP (mmol/I)	2.50±0.16	2.58±0.19	**0.046**
CRP (mg/dl)	6.93±1.30	6.83±1.37	0.730
Fibrinojen (mg/dl)	280±13	284±6	**0.045**
B2 Mikroglobulin (mg/L)	2.60±0.14	2.57±0.13	0.190
IL-1 (pmol/L)	11.80±0.58	11.54±0.53	**0.029**
IL-6 (pmol/L)	13.30±0.73	13.35±0.94	0.770
TNF-alfa (pmol/L)	12.90±0.53	12.93±1.09	0.220
ROS (µmol/L)	18.81±0.49	18.97±0.62	0.830

AOPP: Advanced oxidation protein products. CRP: C-reactive protein. IL: interleukin. TNF- α: tumor necrosis factor-alpha. ROS: Reactive oxygen species

and white blood cells count. In our study, there was no significant correlation between AOPP levels and CRP. In another study performed on peritoneal dialysis patients,[14] no correlation was found between AOPP and CRP, IL-6 and TNF alpha as in our study.

Although ROS levels were similar in both groups in our study, AOPP levels were higher in patients receiving intravenous iron treatment (Table 2). Moreover, ROS was found to be an independent predictor of AOPP. Based on these findings, it may be said that intravenous iron treatment increases AOPP levels via ROS. Intravenous iron therapy may lead to increased oxidative stress. This may be explained by formation of hydroxyl radicals which are more toxic than superoxide and hydrogen peroxide.[6,7,15] There was no significant correlation of AOPP levels with iron and ferritin levels in our study. Drueke et al.[15] detected previously a significant correlation between AOPP and ferritin. These different findings may be related to methodological differences regarding the timing of blood sampling. As is well known, markers of oxidative stress decrease as free iron disappears from blood. Since the acute effects of intravenous iron infusion vanishes within two weeks, we analyzed blood samples of patients obtained two weeks after the last intravenous iron dose. In the study of Drueke et al.[15] samples were taken without a period free of intravenous iron.

The mean transferrin saturation was found to be 25.4% and was within the target levels proposed by international nephrology guidelines (The European Renal Best Practice, Kidney Disease Outcomes Quality Initiative). Although no relationship was found between AOPP, iron indices and inflammatory markers (CRP, IL-1, IL-6, TNF-α and fibrinogen). AOPP levels were higher in patients receiving intravenous iron therapy.

Iron formulations that are currently available for intravenous use are known to have potential for lipid peroxidation. It is believed that intravenous iron preparations used in the modern era have carbohydrate sheath (iron dextran, iron sucrose) which attenuates oxidant effects of iron.[15] This fact together with the finding that transferrin saturation was within target levels may be possible explanations for the lack of correlation between iron indices, oxidative stress and inflammation.

Malnutrition-inflammation-atherosclerosis (MIA) syndrome has been reported to be present in 18-75% of HD patients in different studies. The hypothesis of MIA syndrome that was first described by Stenvinkel et al.[9] in

2000 is based on the relationship between malnutrition, accelerated atherosclerosis and increased levels of proinflammatory cytokines. Morbidity and mortality of patients with ESRD increase and life expectancy decreases as the number of components of MIA syndrome increase. A positive correlation has been detected between AOPP levels and carotid intima media thickness that is accepted as an early sign of atherosclerosis[15]. Serum CRP, IL-6 and TNF-α are among the most important markers of inflammation. We also detected increased levels of them in our study. Moreover, in our study, the relationship between TNF-α and CRP and between IL-6 and creatinine levels were statistically significant with multivariate analysis.

ESRD is a chronic inflammatory process. Serum CRP, TNF-α, serum amyloid-A and IL-6 are used frequently as inflammatory markers. Patients with ESRD are known to have serum proinflammatory cytokine levels 10 times higher than the healthy population. Inflammation was found in 35-60% of patients on HD. while this ratio was lower in peritoneal dialysis patients.[16] Possible etiological factors that may be important for this proinflammatory state include persistent infections including those of the oral cavity, periodontitis, decreased clearance of cytokine, graft or fistula infections in HD patients, bioincompatible dialysis solutions and membranes.[17] We detected increased levels of CRP, IL-1, IL-6 and TNF-α levels in our study population.

Erythropoietin resistance is more frequent in patients with inflammation, and is related with decreased survival.[17] Serum albumin level is useful in evaluating malnutrition in patients with ESRD. Its synthesis decreases significantly in the presence of inflammation. It has been reported that erythropoietin increases muscle catabolism by way of stimulating ketoacid dehydrogenase. Increased proinflammatory cytokines increase protein catabolism by ubiquitin proteasome pathway, and decrease albumin synthesis.[16]

Oxidative stress adds to atherosclerosis. It has been reported that the most important reasons for oxidative stress in HD patients are inflammation and long duration of dialysis. Oxidative stress increases the amount of lipid peroxidation products and advanced oxidation products.[17] The mean albumin level was found to be 3.52±0.23 g/dl in our study. Albumin was found to be negatively correlated with IL-6 level (r=-0.219, p=0.01).

Conclusions

Although serum AOPP level was higher in patients on intravenous iron treatment, it was not correlated with iron indices and inflammatory markers. So, intravenous iron may exert its oxidant effect free from serum iron indices. There is need for prospective randomized controlled studies in which the changes in the markers of oxidant stress and proinflammatory cytokines after intravenous iron are evaluated.

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