

# Relation of Antioxidants and Acute-Phase Reactants in Patients Receiving Hemodialysis

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**Introduction.** To investigate the oxidative stress and its association with antioxidants in patients on hemodialysis, we evaluated the levels of albumin, C-reactive protein (CRP), ferritin, vitamin E, glutathione, and total antioxidant capacity in these patients.

**Materials and Methods.** In a cross-sectional study, we enrolled 25 patients on maintenance hemodialysis and measured the inflammatory and oxidative stress indicators consisting of the plasma concentrations of glutathione, vitamin E, and total antioxidant capacity. The acute-phase inflammatory response was assessed by determining the serum levels of CRP and albumin as well as the plasma level of ferritin. Antioxidants and acute-phase reactants in men and women and their association with age and their correlations with each other were analyzed.

**Results.** The mean age of the patients was  $53.6 \pm 14.1$  years (range, 29 to 70 years). They had been on hemodialysis for a mean duration of  $4.66 \pm 5.08$  years. There were no association of sex or age with the levels of antioxidants and acute-phase reactants. Plasma level of glutathione significantly correlated with CRP ( $r = 0.48$ ;  $P = .01$ ) and serum albumin ( $r = 0.42$ ;  $P = .04$ ). Duration of dialysis did not correlate with the antioxidants or acute-phase reactants.

**Conclusions.** Although it is reasonable to see an association between acute-phase reactants and levels of antioxidants in patients on hemodialysis, we failed to show such a relation. It is recommended that other biomarkers of oxidative stress and their relation in patients with kidney failure be investigated.

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## INTRODUCTION

Factors such as exposure of the blood to dialyzer membranes and high risk of acute and chronic infections make patients on hemodialysis susceptible to oxidative stress. Diet limitations in the intake of the antioxidant vitamins in the diet of end-stage renal disease (ESRD) patients are of another constrains.<sup>1</sup> A handful of the recent studies have confirmed the presence of the signs

related to oxidative stress in patients receiving hemodialysis.<sup>2-5</sup> Dysregulated inflammatory response and metabolic derangements in ESRD patients are the predisposing factors of oxidative stress.<sup>6</sup> On the other hand, the level of antioxidant agents such as glutathione and the activity of glutathione peroxidase and glutathione reductase are decreased in patients with chronic kidney disease (CKD) compared with those in healthy

individuals.<sup>7</sup> Owing to the paucity of studies on the effects of dialysis on oxidative stress parameters and their relation with antioxidants, we decided to design this investigation to evaluate the levels of oxidative stress indicators (albumin, C-reactive protein [CRP], and ferritin) and antioxidants (vitamin E, glutathione, and total antioxidant capacity [TAC]) and their correlations in patients on maintenance hemodialysis.

## MATERIALS AND METHODS

In a cross-sectional study, we enrolled 25 patients with ESRD who were on maintenance hemodialysis from 2005 to 2006 at Imam Khomeini Hospital in Tehran, Iran. Patients on hemodialysis for at least 3 months were eligible, and diabetic patients and those who were receiving vitamin C were excluded. All of the enrolled patients were on dialysis for 4 hours, 3 times a week, using bicarbonate-containing dialysis solution. The dialysis flow rate was 200 mL/min to 300 mL/min, and vitamin E, 100 mg/d, was administered for all of the patients.

We measured the inflammatory and oxidative stress indicators consisting of the plasma concentrations of glutathione, vitamin E, and TAC. A blood sample from the peripheral veins was taken and sent in heparinized containers to the laboratory. Measurement of glutathione was performed by Tietze method in which serum glutathione is oxidized by dithio-bis-2-nitrobenzoic acid and is reduced by glutathione reductase. Plasma glutathione was monitored based on the 5'-thio-2-nitrobenzoic acid formation volume at 412 nm frequency and was determined according to the standard curves.<sup>8</sup> Plasma concentration of vitamin E was measured using the high-performance liquid chromatography method.

The acute-phase inflammatory response was assessed by determining the serum level of CRP in a high-sensitive approach and a value greater than 10 mg/L was considered abnormal. Also, using the spectrophotometry (immunoturbidometry), the plasma level of ferritin was measured and levels higher than 100 ng/mL was considered abnormal. Finally, serum albumin was determined by spectrophotometry and levels lower than 4 g/L was considered abnormal.

Data were collected and the levels of antioxidants and acute-phase reactants in men and women and their association with age and their correlations with each other were analyzed. The Mann-Whitney *U* test was used to compare quantitative variables and Spearman correlation test to evaluate bivariate correlations.

## RESULTS

Of 25 patients, 18 (72%) were men and 7 (28%) were women. Their mean age was  $53.6 \pm 14.1$  years (range, 29 to 70 years). Six patients (24%) were 21 to 40 years of age, 12 (48%) were 41 to 60 years, and 7 (28%) were older than 60 years. They had been on hemodialysis for a mean duration of  $4.66 \pm 5.08$  years (range, 0.4 to 15 years).

The values of antioxidants and acute-phase reactants are depicted in Table 1. There were no association between age and levels of antioxidants and acute-phase reactants. Also, men and women were not different in most measured biomarkers (Table 1). Plasma level of glutathione significantly correlated with CRP ( $r = 0.48$ ;  $P = .01$ ) and serum albumin ( $r = 0.42$ ;  $P = .04$ ), and the other antioxidants and acute-phase reactants were not associated with each other (Table 2). Duration of dialysis was not an influencing factor for the antioxidants or acute-phase reactants.

**Table 1.** Antioxidants and Acute-Phase Reactants in Patients on Maintenance Hemodialysis\*

Variable	All Patients	Men	Women	<i>P</i>
<b>Antioxidants</b>				
Vitamin E, mg/dL	19.53 ± 7.33	19.88 ± 7.95	18.49 ± 5.71	.89
Glutathione, μmol/L	64.07 ± 20.44	67.51 ± 21.90	55.22 ± 13.63	.11
TAC, (mmol Trolox Equiv/L)	1.00 ± 0.15	0.97 ± 0.13	1.06 ± 0.21	.19
<b>Acute-phase reactants</b>				
Albumin, g/L	4.15 ± 0.38	4.24 ± 0.38	3.91 ± 0.30	.048
CRP, mg/L	41.10 ± 77.57	51.76 ± 89.27	33.69 ± 16.55	.85
Ferritin, ng/mL	728.76 ± 555.78	858.28 ± 604.24	395.73 ± 146.20	.16

\*TAC indicates total antioxidant capacity and CRP, C-reactive protein.

**Table 2.** Correlations Between Antioxidant and Acute-Phase Reactants in Hemodialysis Patients\*

Antioxidants	Acute-Phase Reactants		
	CRP	Albumin	Ferritin
Vitamin E	0.30 (.20)	0.08 (.74)	-0.06 (.82)
Glutathione	0.48 (.01)	0.42 (.04)	0.10 (.63)
TAC	-0.07 (.79)	-0.44 (.10)	-0.05 (.84)

\*Values are correlation coefficients and those in parentheses are *P* values. TAC indicates total antioxidant capacity.

## DISCUSSION

Oxidative stress is a persistent manifestation of CKD which has been documented by the increase in the oxidative products of fat, carbohydrates, and proteins in the tissue and plasma of uremic patients. Several etiologic factors for oxidative stress have been proposed in these patients, including uremia, reaction of the blood with dialyzer membranes and subsequent contaminating with dialysis byproducts, acute or chronic bacterial infections, parenteral administration of ferrous sulfate, limitation in consumption of fruits and vegetables (that are reached with antioxidant vitamins and natural phytochemicals) in the patients' diet, and underlying diseases.<sup>2,9,10</sup> Oxidative stress in patients on hemodialysis is accompanied by several risks. It has been shown that it can lead to coronary artery disease (CAD),<sup>10</sup> which is the most important cause of mortality and morbidity in ESRD patients.<sup>11</sup> Furthermore, oxidative stress is responsible for alterations in the composition of lipoproteins that result in oxidation of low-density lipoproteins and acceleration of atherosclerosis process.<sup>11</sup> Finally, it seems that reactive oxygen species (ROS) has a role in the pathophysiology of ischemia-reperfusion injury. Due to the impact of ROS on the regulation of cellular cycle, oxygen radicals may cause hypertrophy of the renal tubular cells.<sup>12</sup> It has been demonstrated that the formation of ROS has some effects on the development of kidney diseases, such as glomerulonephritis, acute or progressive kidney failure, proteinuria, and tubulointerstitial nephritis.<sup>13,14</sup> On the other hand, oxidative stress in CKD has an important role in the pathogenesis of hypertension (oxidation of nitrite oxide, arachidonic acid and endothelial changes), neurological disorders (nitration of cerebral proteins and oxidation of myelin), anemia (reduction of erythrocytes' lifespan), inflammation (nuclear factor kappa B activation), fibrosis, and apoptosis.<sup>1</sup>

Today, there is no doubt that correction of the oxidants/antioxidants imbalance in ESRD patients can lower the risk of heart diseases to a great extent.<sup>10</sup> Treatment with antioxidants can prevent CAD in patients on hemodialysis, given the increased oxidative stress in these patients. In 2 studies a positive effect of vitamin E on lipid metabolism, atherosclerosis, and CAD in hemodialysis patients.<sup>15,16</sup> However, no document has been presented in favor of its effect on the clinical end-point or the overall mortality.

The present study was conducted to assess the antioxidants levels and their relation with acute-phase reactants in patients on maintenance hemodialysis. We found no sex- or age- related differences in these factors. The only significant correlation was observed between the plasma level of glutathione and the CRP and albumin serum levels. While ESRD patients are susceptible to infections, the ROS formation is a part of nonspecific immune response against microbial microorganisms.<sup>17</sup> Reduction of the serum levels of these proteins such as albumin is suggestive of an inflammatory response.<sup>18</sup> Thus, it is reasonable to see an association between acute-phase reactants and levels of antioxidants. To our knowledge, there is no study on such relations in hemodialysis patients. However, no association has been found between these variables in other study populations. Bergesio and colleagues showed that measurement of TAC is not a reliable method for assessment of oxidative stress in patients with CKD.<sup>19</sup> In a study of patients on hemodialysis and peritoneal dialysis, Samouilidou and associates found no relation between CRP and TAC or between CRP and malonyldialdehyde.<sup>20</sup>

Although we found a correlation of glutathione with albumin and CRP, it is mostly probable that some other factors influence the acute-phase reactants and antioxidants acting independently. This explains the lack of association or weak correlations observed in the abovementioned articles. In addition, there might be associations between other antioxidants such as vitamin C and the acute-phase reactants. Humenikova and colleagues found that the antioxidants serum levels and risk factors of CAD are good predictors of the CRP serum level.<sup>21</sup> It has been demonstrated that a close relationship does exist between the oxidative stress levels and high-sensitive CRP, while there

is no association with other derivatives of CRP.<sup>22</sup> Also, it is depicted that in hemodialysis patients, the inflammation extent and the levels of oxidative stress biomarkers are higher among those with hypoalbuminemia. Accordingly, it can be concluded that hypoalbuminemia, acute-phase inflammation, and oxidative stress act synergically as factors that increase the risk of mortality as factors that increase the risk of mortality and morbidity resulting from CAD.<sup>23</sup> Annuk and coworkers showed that the CRP level correlates with lipid peroxidase in CKD.<sup>24</sup> While the endothelial function is related to intracellular oxidative stress, no association was observed between CRP and endothelial-depending vasodilation. Thus, CRP and endothelial function can have complementary prognostic value for coronary artery disorders in patients with CKD.

## CONCLUSIONS

We found no association of sex or age with the antioxidants and acute-phase reactants levels in patients on maintenance hemodialysis. The only significant correlation was observed between the plasma level of glutathione and the CRP and albumin serum levels. Although it is reasonable to see an association between acute-phase reactants and levels of antioxidants, we failed to show such a relation. To our knowledge, there is no study on such relations in hemodialysis patients. It is recommended that other biomarkers of oxidative stress and their relation be investigated.

## CONFLICT OF INTEREST

None declared.

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