Oxidative Stress Markers and C-Reactive Protein in Pediatric Patients on Hemodialysis

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Total antioxidant capacity · Lipid peroxidation product · High-sensitivity C-reactive protein · Hemodialysis

Abstract

Background: Pediatric patients with end-stage renal disease undergoing hemodialysis (HD) are exposed to oxidative stress associated with an impairment of antioxidant defense and an overproduction of oxidative stress markers. Oxidative stress plays a significant role in the development of inflammation in these patients. Objectives: The high incidence of cardiovascular disease in HD pediatric patients is now well established and the involvement of oxidative stress has been hypothesized. This study focuses on a comparison of plasma total antioxidant capacity (TAC) and lipid peroxidation product and evaluates the relationship between these parameters and high-sensitivity C-reactive protein (hsCRP) in pediatric patients on HD. Subjects and Methods: Plasma TAC, lipid peroxidation products, malondialdehyde (MDA) as well as hsCRP were determined in 30 pediatric patients on HD and in 20 healthy controls (HC). Results: TAC and MDA levels were significantly higher in children on HD than in the HC (p < 0.001). The hsCRP values were also significantly higher in HD patients than in HC (p < 0.001). The percentage of HD pediatric patients with CRP >10 mg/l was 30%. The concentrations of TAC and MDA correlated positively with hsCRP in HD patients (TAC: r = 0.52, p < 0.08; MDA: r = 0.75, p < 0.04), but not in HC. Conclusion: Our study demonstrates an increase in oxidative stress in children on HD and that the susceptibility to oxidative stress is strongly related to the levels of MDA produced in plasma. hsCRP levels are higher in children on HD than in HC and this is indicative of a higher degree of inflammatory activity in the former patients. These profound disturbances in oxidative stress markers may provide an explanation for the cardiovascular complications in HD patients.

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Introduction

Cardiovascular disease is the major cause of morbidity and mortality in patients with end-stage renal disease receiving renal replacement therapy [1]. Several factors may contribute to the greatly increased risk of atherosclerotic disease, including an increased prevalence of hypertension. In addition, patients with renal failure have an abnormal lipid profile characterized by reduced high-density lipoprotein cholesterol and moderate hypertriglyceridemia. However, these factors by themselves are not sufficient to account for the excess prevalence of cardiovascular disease observed [2].

Patients on hemodialysis (HD) are constantly exposed to oxidative stress [3–5]. This is mostly attributed to biocompatibility-type reactions, originating from the di-
alysis membrane and the imbalance between oxidants and antioxidants due to the diffusion of hydrophilic compounds into the dialysate. The intensity of oxidative stress in HD patients can be influenced by many factors among which are the duration of dialysis therapy, the primary cause of chronic renal failure, the intensity of chronic inflammation, the type of diet and environmental toxins [6–8].

One of the events that predominate in oxidative stress is the alteration of lipid metabolism, which is demonstrated by the oxidation of low-density lipoprotein plasma lipids and the release of short-chain aldehydes such as malondialdehyde (MDA) [9–11]. The increase in lipid peroxidation (LPO) in HD patients accompanied by abnormal antioxidant defenses has pathologic relevance to many diseases, in particular to those having an inflammatory component, such as atherosclerosis [7, 12–15]. Thus, it appears that in HD therapy a synergism between oxidative stress and inflammatory response is developed. Plasma C-reactive protein (CRP) is considered a sensitive marker of induction of inflammatory activity in all patients treated with HD [13, 16, 17].

The present study attempts to look into a comparison between high-sensitivity CRP (hsCRP) levels and the serum levels of both total antioxidant capacity (TAC) and LPO product in HD patients to find a possible explanation for the increased cardiovascular risk in these children.

**Patients and Methods**

Thirty patients with end-stage renal disease on regular HD therapy were selected from the HD unit of the Center of Pediatric Nephrology and Transplantation (CPNT), Children’s Hospital, Cairo University, were included in this study. The study was done from December 2007 to April 2008. The examined patients were 17 (56.67%) males and 13 (43.33%) females (age: 10.53 ± 3.43 years; range: 4–18 years). They were treated with HD for 3–4 h thrice weekly with a polysulphone membrane using bicarbonate-buffered dialysate produced with ultrapure water of high bacteriological quality. The blood flow rate ranged from 80 to 180 ml/min according to body weight, the dialysate flow rate was 500 ml/min and was not changed, and heparin was used as anticoagulant.

Inclusion criteria comprised children on regular HD treatment for at least 3 months. None of the patients had evidence of systemic infection (fever or leukocytosis). Patients with known cardiovascular disease, chronic hepatitis, hematological and inflammatory disorders were excluded. Their underlying renal disorders were reflux nephropathy disease (n = 1; 3.33%), glomerular disease (n = 5; 16.67%), hereditary causes (n = 6; 20%), anatomic causes (n = 9; 30%) and unknown causes (n = 9; 30%). All patients received recombinant human erythropoietin at a dose of 500–12,000 IU/week.

Twenty subjects [10 (50%) male, 10 (50%) female; average age: 10 ± 1.07 years; range: 3–17 years] were recruited as healthy controls (HC). The parents of all the patients and controls included in this study gave their informed consent.

All patients were subjected to full history taking and thorough clinical examination. Measurement of HD adequacy was as follows: the delivered dose of HD was described as the fractional clearance of urea as a function of its distribution volume (Kt/V) and was determined by using the Kt/V natural logarithm formula [18].

Serum from all patients on HD was obtained by collecting 5 ml whole blood before dialysis. The serum was immediately centrifuged for 10 min at 5,000 rpm at 4 °C and transferred into sterile tubes. All samples were stored at −70 °C until the assay was started.

Complete blood count, pre- and postdialysis kidney function test and serum albumin estimation were determined by standard laboratory methods. Estimations of the plasma concentration of uric acid and cholesterol were made by using an Olympus AU400 which is an automated clinical chemistry analyzer (Olympus America, Inc., Center Valley, Pa., USA). The assay of TAC was performed using a colorimetric assay. TAC was determined by an enzymatic reaction which leads to the conversion of 3,5-dichloro-2-hydroxybenzenesulfonate to a colored product (Biodiagnostics, Egypt) [19]. The assay of MDA was done by spectrophotometry. This assay was based on the reaction of carbonyl complexes that can be measured according to the method of Draper and Hadley [20]. The reagents were thiobarbituric acid (Merck, Darmstadt, Germany) and trichloroacetic acid (SISCO Research Laboratories, Pvt. Ltd, Mumbai, India). The determination of hsCRP in serum was performed by solid-phase chemiluminescent immunometric assay (Immulite/Immulite 1000; Siemens Medical Solution Diagnostics, Eschborn, Germany) [21].

**Statistical Analysis**

SPSS (statistical package for social sciences) version 11.0 was used in data analysis. Histograms and normality plots were used for evaluating the normality of data. For those data with abnormal distribution, log transformation was performed before a t-test. Data were summarized as mean ± SD, range and percentage. Independent t test for quantitative independent variables was used for the analysis of difference between the 2 groups. Pearson’s and Spearman’s rho correlation analysis were performed to predict the associations between TAC, MDA and CRP as well as other numerical variables. Multiple linear regression analysis was performed to determine the contribution of various factors as independent or covariates to TAC, MDA or CRP as the dependent variables. p < 0.05 was considered significant.

**Results**

Demographic and clinical data of the groups studied are shown in table 1. There were no significant differences in age or gender between HD patients and controls.
The hematological data and the biochemical profiles of the patients and the healthy individuals are shown in table 2. The concentrations of antioxidant uric acid and creatinine were significantly increased in HD patients when compared to the HC ($p < 0.01$). There was a statistically significant difference between HD patients and HC as regards cholesterol concentration ($p < 0.01$).

The plasma concentration of TAC measured in renal patients on HD ($2.24 \pm 0.64 \text{ μmol/l}$) was significantly higher than in the HC ($0.16 \pm 0.07 \text{ μmol/l}$) ($p < 0.001$). The concentration of the LPO product MDA was significantly increased in the serum of HD patients ($2.05 \pm 0.39 \text{ μmol/l}$) compared to the HC ($1.01 \pm 0.41 \text{ μmol/l}$) ($p < 0.001$; fig. 1).

A comparison between the values of hsCRP in the serum of HD patients and in the control subjects is shown in figure 2. The hsCRP values ($6.57 \pm 5.57 \text{ mg/l}$) were higher in HD patients than in the HC ($0.19 \pm 0.03 \text{ mg/l}$).
Raised hsCRP concentrations (>10 mg/l) were found in 30% of the HD patients. Patients with levels of hsCRP >10 mg/l did not show significant differences in TAC and MDA concentrations from patients who had hsCRP levels within normal range.

Positive correlations were observed between hsCRP and both TAC concentrations and MDA levels in the serum of HD patients (TAC: r = 0.52, p < 0.08; MDA: r = 0.75, p < 0.04) (table 3). No correlation was found between hsCRP and TAC concentrations or hsCRP and MDA levels in the serum of the HC.

The correlations of oxidative stress markers and hsCRP with individual variables in HD patients are shown in table 4. TAC concentration was positively correlated with uric acid levels (r = 0.66, p < 0.002) as well as with predialysis urea levels (r = 0.35, p < 0.08). hsCRP values were inversely correlated with cholesterol concentration in the serum of HD patients (r = −0.35, p < 0.03). An inverse correlation was found between serum MDA and hemoglobin (Hb) concentrations in blood of HD patients (r = −0.42, p < 0.01).

On correlating the hsCRP values to oxidative stress markers and other individual variables by multiple linear regression analysis, cholesterol concentration, Hb and equilibrated Kt/V were variables that were independently associated with elevated hsCRP values (p < 0.05). The serum concentration of TAC was independently associated with hsCRP levels, cholesterol concentration and Hb levels (p < 0.05), while MDA levels were independently associated with TAC concentration, Hb values and albumin concentration in HD patients (p < 0.05) (table 5).

**Discussion**

Chronic uremia is considered a proinflammatory state associated with high cardiovascular morbidity and mortality [22]. Patients treated by renal replacement therapy...
are subjected to a wide range of biochemical disorders, some of which are ascribed to increased oxidative stress [3, 4, 8, 13]. Our finding that serum concentrations of TAC were elevated in HD patients was consistent with this fact. There is currently a great interest in the assessment of antioxidant status as antioxidant depletion may contribute to a number of diseases. Several methods reported in recent years provide a single measure of the TAC of plasma, and it has been suggested that this may represent a useful way of predicting the risk of free-radical-induced tissue damage [23]. Methods of measuring TAC are attractive in several ways: a small sample volume is required, the interactions between individual antioxidants, and the effects of unknown antioxidants are taken into account [24]. This result is in agreement with Samouilidou et al. [25] who reported an increased level of TAC concentration in patients on peritoneal dialysis and in patients on HD. Moreover, Jackson et al. [26] found that TAC levels were increased in HD patients who are susceptible to oxidative stress.

In the present study, there was an increased concentration of MDA in the dialysis patients as compared to the HC, suggesting an increase in the LPO and the formation of aldehydic products [25]. This is in keeping with most findings previously published. Thus, Samouilidou and Grapsa [27] found that, before HD, TAC and MDA levels were higher in patients than in HC. After HD, these levels decreased significantly but were still higher than in the HC.

**Table 5.** Multiple linear regression analysis comparing the correlation of hsCRP levels and markers of oxidative stress with individual variables in serum of HD patients

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>β</th>
<th>p</th>
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<tbody>
<tr>
<td>hsCRP</td>
<td></td>
<td></td>
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<tr>
<td>Cholesterol</td>
<td>-0.48</td>
<td>0.06</td>
</tr>
<tr>
<td>Hb</td>
<td>-0.49</td>
<td>0.07</td>
</tr>
<tr>
<td>Kt/V</td>
<td>-0.72</td>
<td>0.01*</td>
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<tr>
<td>TAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsCRP</td>
<td>0.35</td>
<td>0.02*</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.73</td>
<td>0.001*</td>
</tr>
<tr>
<td>Hb</td>
<td>0.42</td>
<td>0.01*</td>
</tr>
<tr>
<td>MDA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAC</td>
<td>0.51</td>
<td>0.03*</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.42</td>
<td>0.07</td>
</tr>
<tr>
<td>Hb</td>
<td>-0.35</td>
<td>NS</td>
</tr>
</tbody>
</table>

* p < 0.05.

In our study, hsCRP levels were increased in the HD patients when compared to the HC. It is thought that this marker may be an independent risk factor that may cause progressive atherosclerosis [13, 16, 17]. As is known, one of the mechanisms that may account for increased oxidative stress in dialysis is chronic inflammation. CRP is an excellent marker of systemic inflammation. The release of proinflammatory cytokines such as interleukin-1, interleukin-6 and tumor necrosis factor-α during dialysis sessions is considered a reason for elevated plasma CRP levels. Reduced renal clearance, undetected persistent infections, dialysis-related factors, comorbidity (such as chronic heart failure) and accumulation of advanced glycation end-products are various factors that cause a chronic inflammatory status in uremic patients [8]. The inappropriate cytokine generation and activity during HD can be related to contaminated dialysate, direct contact of peripheral mononuclear blood cells with the dialysis membrane or activated complement fractions. Dialysate contamination is a relevant factor triggering monocyte/macrophage activation. Therefore, the assumption can be made that it is crucial to improve water dialysate purity. Biocompatible membranes may induce inappropriate monocyte activation and cytokine production [10]. The ability of monocyte-derived macrophages to secrete cytokines, chemokines (MCP-1) and growth factors may lead to the activation and proliferation of smooth muscle cells and the progression of vascular lesions, suggesting a link between CRP, cytokines, inflammation and coronary artery disease [28].

It has been shown that hsCRP levels of >50 mg/l are strongly suggestive of an inflammatory process while a level of <10 mg/l may exclude it [15, 16]. The present study showed that the percentage of children on HD with hsCRP >10 mg/l was 30%. This is in agreement with a previous study [13] where it was assumed that HD patients are characterized by a higher inflammatory activity that may probably lead to a chronic state of acute phase response [28, 29]. In the literature, a wide range of hsCRP levels in chronic renal failure patients are reported. Zimmermann et al. [17] reported that an increase in hsCRP levels to >10 mg/l occurred in 46% of HD patients, while Owen and Lowrie [30] reported the same increase in only 35% of their cases.

The present study showed a positive correlation between hsCRP levels and TAC concentration. Differences in inflammatory activity in HD patients may be responsible for this correlation [24]. hsCRP levels were proved to be correlated with the levels of specific components of the antioxidant defense system such as α-tocopherol (ex-
pressed in lipid-normalized values) [7]. This is in agreement with the results of Nguyen-Khoa et al. [7].

It is also of interest to note that hsCRP concentrations correlated positively with MDA values in this study. Donica [10] reported that CRP levels were shown to be associated with another product of LPO: esterified F2-isoprostanes. This might be suggestive of a possible selective stimulation of CRP release by peroxyl radicals produced by oxidized lipids under conditions of oxidative stress.

Our study showed a positive correlation between TAC concentration and uric acid levels in HD patients. This is in accordance with the results of Jackson et al. [26] who reported that serum urate was increased as expected given the key role of the kidney in the elimination of urate from the body. Total antioxidant content was increased almost entirely due to increased urate. Our results highlight one pitfall of measuring TAC: changes in one of the major contributors (in this case, urate) may mask potentially important changes in other antioxidants. Urate is an efficient antioxidant in some settings, particularly against ozone-derived radicals. However, it is not a good scavenger for some other biologically important radical species, and increased urate concentrations are unlikely to provide an adequate antioxidant defense in the presence of deficiencies of other antioxidant systems [31, 32]. We therefore believe that it is important to measure the major chain-breaking antioxidants individually in addition to TAC levels.

The present study showed an inverse correlation between MDA serum concentrations and Hb levels in blood of HD patients. Lucchi et al. [33] found an inverse correlation between an LPO product and the Hb in blood of HD patients. This might indicate 2 or more different mechanisms leading to the dependence shown. Besides the effect of reduced RBC survival due to uremia [34], the accelerated LPO at low Hb levels might be explained by oxidative stress due to the anemic condition itself. Anemic patients showed an increased frequency of ventilation at peak exercise because of the limited oxygen transport capacity, implying an anaerobic metabolism due to hypoxemia and ischemia [35]. There are important radical sources that may be responsible for oxidative stress in anemic HD patients: final purine degradation via xanthine oxidase reoxygenation of the temporarily hypoxic tissue, activation of polymorphonuclear lymphocytes and partial uncoupling of oxidative phosphorylation [36, 37]. On the other hand, since RBC deficiency accompanies a deficit of enzymes able to metabolize aldehydic LPO products, the blood of uremic patients loses a major part of its antioxidant power [38].

The present study showed an inverse correlation between hsCRP levels and cholesterol concentrations in children on HD. This result may be due to the coexistence of malnutrition and inflammation, which is a common finding in patients of HD [25]. A possible mechanism might include increased uptake of cholesterol by oxidized macrophages via oxidized cholesterol receptor. Observed in vitro, this effect might be attributable to an increased number of cholesterol receptors on the surface of oxidized macrophages [39]. This result is in agreement with the result of Zimmermann et al. [17] who reported that hsCRP levels were proved to be negatively correlated with the levels of total cholesterol in HD patients.

In the present study, independent associations were revealed between hsCRP levels in the serum of HD patients and cholesterol concentrations, Hb concentrations and equilibrated Kt/V by multiple linear regression analysis. This result indicates that CRP in HD patients correlates with some nontraditional risk factors for atherosclerosis. This suggests that CRP levels are increased in children on HD as a result of severe oxidative stress related to anemia, malnutrition and dyslipidemia [1].

We also found that hsCRP levels, cholesterol concentrations and Hb values were independent predictors for increased TAC concentrations in pediatric patients on HD. In an interesting study to detect the role of TAC in sepsis, Chuang et al. [40] demonstrated that the increase in TAC levels was directly correlated with severity of illness and poor outcome. Although the increase in TAC might be interpreted as an extreme protective attempt against overwhelming inflammation, this must still be proved.

When modeled, MDA levels were independently associated with TAC concentrations, Hb values and albumin. It is suggested that HD patients in greater oxidative stress are at greater risk of cardiovascular disease [3]. Carluccio et al. [41] reported that MDA is independently associated with malnutrition in patients with chronic renal failure. The close association of MDA levels with Hb concentrations revealed that a substantial part of the oxidative stress is due to renal anemia [33].

Vitamin E is an antioxidant agent, but it is also known to have prooxidative action under special conditions that can be encountered in HD patients [42]. α-Tocopherol administration induces a significant decrease in TAC levels. Prolonged oral α-tocopherol administration in HD patients induces a decrease in some components of the antioxidant defense system, raising the possibility of a prooxidative role of vitamin E.
Conclusions

Our data showed that in spite of an increased antioxidant capacity in HD patients, there is an increased susceptibility to oxidative stress which is strongly related to the serum levels of MDA produced in the serum. hsCRP levels were higher in HD patients than in HC and this is indicative of a higher degree of inflammatory activity in HD patients. A synergism between oxidative stress and inflammation in HD patients may result in the accelerated atherosclerosis observed in these patients. A substantial part of the oxidative stress is due to renal anemia.

Based on these conclusions, the following recommendations can be made:

1. The identification of MDA as a predictor of cardiovascular disease in HD patients may underscore the role of oxidative stress as a cardiac risk factor in a pediatric patient population.

2. A clinical trial in which antioxidants could be used to reduce oxidative stress in this group is required. Supplementation with vitamin E may be a promising antioxidant intervention in these patients.

3. Further investigation on diverse mechanisms of increased oxidative stress induced by CRP elevation in HD patients would enlighten the possible pathways that lead to the generation of atherosclerosis.

References


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