OXIDATIVE STRESS AND ANTIOXIDANTS IN CHRONIC RENAL FAILURE WITH AND WITHOUT DIALYSIS

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ABSTRACT: The present study was designed to demonstrate oxidative stress in chronic renal failure (CRF) patients with the progression of the disease. Plasma advanced oxidative protein products (AOPP), a novel marker of oxidative damage to proteins and % hemolysis an indirect marker of lipid peroxidation were estimated in CRF patients with and without dialysis. Antioxidant status was evaluated by determining GSH, total thiols, albumin and total antioxidant activity in plasma. All the parameters were estimated by spectrophotometric methods. Data was compared with that of age and sex matched controls. AOPP increased significantly in plasma of CRF patients along with %hemolysis compared to controls. % hemolysis significantly increased in hemodialysis patients compared to nondialysed CRF patients. Exhaustion of antioxidants in plasma was evident with significant decrease in albumin, GSH and total thiols in CRF patients as compared to healthy controls. Globulin levels increased significantly in these patients. Plasma albumin decreased significantly with the progression of renal failure. The oxidant: antioxidant imbalance may contribute to the development of pathogenic changes in chronic renal failure.

Key words: Protein oxidation, Total antioxidant activity, GSH, Renal failure

INTRODUCTION

The most common conditions associated with reactive oxygen species (ROS) toxicity are chronic diseases like atherosclerosis, diabetic nephropathy, lung fibrosis, cancer and different glomerulopathies [1]. Oxidative stress and subclinical inflammation may be crucial factors in pathogenesis of chronic renal disease. Chronic renal failure (CRF) patients suffer from dysregulation of immune system with increase in activated monocyte derived proinflammatory cytokines [2], leading to massive generation of ROS. Recent studies suggest that profound deficiency in antioxidants [3] remain leading cause of morbidity in dialysis patients [4]. In vitro studies have proved the vulnerability of proteins to ROS [5]. In contrast, evidence for the presence of oxidatively modified proteins in vivo and their possible clinical significance is still lacking. Hence, the present study is undertaken to assess the protein oxidative damage in chronic renal disease in patients with and without dialysis and to determine the best biomarker to assess this stress. A novel oxidative stress marker of protein oxidation referred to as AOPP (advanced oxidative protein products) and % hemolysis an indirect marker of lipid peroxidation were estimated in the blood samples of normal subjects and CRF patients with and without dialysis. The antioxidant status was evaluated by analysis of total thiols, GSH, albumin and total antioxidant activity. Plasma globulins were estimated to assess the inflammatory response due to renal failure.

MATERIALS AND METHODS

The study group consisted of 75 subjects which included 25 healthy controls, 25 nondialyzed chronic renal failure patients (Group I) with creatinine clearance of 50-75 ml / min and 25 patients with hemodialysis (Group II) whose creatinine clearance was less than 25 ml/min. Patients suffering from diabetes mellitus, epilepsy, psychiatric disorders or those receiving immunosuppression therapy at the time of blood sampling were excluded from the study. The study protocol was approved by institutional ethical committee and informed consent was obtained from all subjects.
6ml of blood was collected from patients in heparinized vacutainers. After centrifugation at 3000rpm for 10mins, plasma and RBCs were separated. Theseparated RBC washed thrice with saline phosphate buffer and suspended in an equal volume of normal saline to get 50% suspension, for the estimation of % hemolysis by method of Kartha and Krishnamurthy [6] Concentration of AOPP in plasma was estimated by measuring the absorbance in acidic conditions at 340nm in the presence of KI [7]. Plasma total protein and albumin were estimated by Lowry’s method. Estimation of glutathione was done by Ernest Beutler method [8] in which yellow compound formed by the reduction of DTNB by GSH was measured. Total thiols were quantified by Ellman method [9] using DTNB in absolute ethanol as colouring compound. Total antioxidant activity in plasma was determined by Koracevic method [10] where inhibition of colour development with thiobarbituric acid was recorded. Plasma iron was estimated by Kitzes method [11] using αα-dipyridyl as chromogen.

RESULTS

The mean plasma AOPP was significantly higher in chronic renal failure patients both with and without dialysis compared to healthy individuals. An apparent increase in AOPP was seen in patients with advanced stage of renal failure compared to patients without dialysis. Likewise, % hemolysis was also significantly high both in group I and group II compared to normal individuals. Further, a comparison of mean values of group I and II showed a significant increase in the latter (table 1). Plasma GSH values decreased significantly in patients without dialysis and the decrease was much more significant in patients undergoing dialysis compared to normal subjects. Total thiols also declined significantly in patients with chronic renal failure compared to healthy subjects. However, the difference was not statistically significant between group I and group II (table 2). Total antioxidant capacity showed an apparent decrease in CRF patients with and without dialysis compared to controls. Plasma albumin was significantly low in CRF patients than in controls and the level decreased significantly with the progression of renal failure. However, globulin levels increased significantly in both the groups of CRF as compared to controls. Further, the increase was statistically significant when group I and II were compared. Furthermore, the A: G declined markedly in CRF patients compared to healthy individuals. This ratio declined significantly with the progression from early to advance stages of renal failure. There was an apparent decrease in serum iron in both groups of CRF compared to healthy individuals.

Table 1: Comparison of Oxidative Damage Parameters in Renal Failure Patients With and Without Dialysis

<table>
<thead>
<tr>
<th></th>
<th>Control (N=25)</th>
<th>CRF without Dialysis (N=25)</th>
<th>CRF with dialysis (N=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOPP(mmol/dL)</td>
<td>0.12 ± 0.05</td>
<td>0.13 ± 0.021*</td>
<td>0.15 ± 0.01*</td>
</tr>
<tr>
<td>% Hemolysis</td>
<td>5.3 ± 2.82</td>
<td>7.18 ± 5.84*</td>
<td>9.88 ± 6.57*</td>
</tr>
<tr>
<td>Iron(ug/dL)</td>
<td>126 ± 34</td>
<td>117 ± 41</td>
<td>119 ± 51**</td>
</tr>
</tbody>
</table>

*p<0.05,**p<0.001 Significant difference when Group I,II was compared with control

Table 2: Comparison of Antioxidants in Renal Failure Patients with and without Dialysis

<table>
<thead>
<tr>
<th></th>
<th>Control (N=25)</th>
<th>CRF without Dialysis (N=25)</th>
<th>CRF with dialysis (N=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH(mmol/L)</td>
<td>45.03 ± 10.45</td>
<td>32.16 ± 15.4*</td>
<td>28.62 ± 12.9**b</td>
</tr>
<tr>
<td>Total thiols(mmol/L)</td>
<td>0.52 ± 0.12</td>
<td>0.32 ± 0.08**</td>
<td>0.29 ± 0.11**</td>
</tr>
<tr>
<td>Total antioxidant capacity(mmol/dL)</td>
<td>1.00 ± 0.05</td>
<td>0.95 ± 0.06</td>
<td>0.81 ± 0.05</td>
</tr>
<tr>
<td>Albumin(g/dL)</td>
<td>4.64 ± 0.8</td>
<td>3.19 ± 0.69**</td>
<td>2.62 ± 0.65**a</td>
</tr>
<tr>
<td>Globulin(g/dL)</td>
<td>3.1 ± 0.6</td>
<td>3.5 ± 0.47*</td>
<td>3.66 ± 0.81**b</td>
</tr>
<tr>
<td>A:G</td>
<td>1.5 ± 0.4</td>
<td>0.92 ± 0.25**</td>
<td>0.72 ± 0.3**b</td>
</tr>
</tbody>
</table>

*p<0.05,**p<0.001 Significant difference when Group I,II were compared with control

*p<0.01,**p<0.05 Significant difference when Group I was compared with Group II
DISCUSSION

It has been stressed recently that oxidative modification of cellular matrix components by oxidants indeed play a major role in permanent renal parenchymal damage [12] in culture medium. ROS are considered to be chemically reactive with biomolecules including DNA, protein and lipids inducing cellular damage and genetic mutations. Oxidative stress and subclinical inflammation are crucial factors in the development of chronic renal disease. In most glomerular nephritis there is an interstitial inflammatory exquisite vulnerability of proteins to ROS which is well documented in invitro studies [13]. In the present study, AOPP significantly increased in CRF patients compared to healthy subjects. The levels gradually rose with the progression of renal failure, emphasized by the fact that there was an inverse relationship between plasma AOPP and GFR [14]. In IgA nephropathy, plasma AOPP was a measure of oxidative stress [15]. Hence the increase in the level not only relates to decrease in kidney excretory function but is also associated with severe inflammatory markers. The significant rise of globulins observed may be secondary to inflammatory response in renal failure and in patients receiving maintenance hemodialysis [4]. Renewed activation of circulating neutrophils and monocytes following blood passage through dialysis circuits leads to massive generation of ROS [4] and release of cytokines [2]. % hemolysis showed a highly significant increase in CRF patients compared to controls, indicative of membrane damage secondary to lipid peroxidation. Lipid peroxidation induces a decrease in erythrocyte GSH and efflux of GSSG, in turn decreasing the life span of RBC in case of people with CRF[16]. Oxidative modification of lipids leads to self perpetuating cycle of ROS production and modification of proteins. The present study also indicates profound defect in antioxidants in CRF patients both with and without dialysis as total thiols and glutathione levels decreased markedly compared to healthy individuals. This adds to the findings of earlier studies in experimental model of glomerulonephritis, which showed a significant decrease in other water soluble, nonenzymatic antioxidants like ascorbic acid and uric acid[17]. A steep decrease in GSH levels in hemodialysis shows further depletion of antioxidants in them. Decrease in GSH in uremic patients may be due to inhibition of G6PD by uremic toxins[18]. Furthermore, oxidation of plasma thiol groups termed as ‘thiol stress’ is quantitatively the major manifestation of protein oxidation [19]. Albumin, a powerful extracellular antioxidant [20] provides 10 fold greater protection against HOCl due to its thiol groups. Hypoalbuminemia with concomittent hyperglobulinemia may be involved in acute phase changes associated with a systemic inflammatory response [2]. While most of the positive acute phase proteins are globulins in plasma, their synthesis in CRF patients may be beneficial. In the present study, plasma total antioxidant capacity showed low both in group I and II compared to healthy subjects. Bergesio et al [21] showed that measurement of total antioxidant capacity is not a reliable marker for the assessment of oxidative stress and in patients with renal failure. However, recent study suggested that antioxidant therapy may lessen cardiovascular complications in CRF patients, suggesting that oxidants may be an important ‘non traditional’ factor [4] in renal pathophysiology.

The protein bound reducing moieties have been shown to be able to reduce transition metals (iron and copper) making them highly pro oxidant molecules. For this reason, careful control of iron availability is crucial to maintain normal nephrotic function. Chronic renal disease is commonly accompanied by development of anemia [22]. Though several reports indicate significant decrease of serum iron in CRF patients [23], present study showed only apparent decrease and the level was steady even in hemodialysis patients probably due to parenteral administration of ferrous sulphate to maintain adequate iron stores.

In conclusion, the present study provides several lines of evidence to suggest that CRF patients are under severe oxidative stress, the magnitude of which magnifies with the advancement of the disease.

REFERENCES


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