Oxidative stress in chronic renal failure patients treated by peritoneal dialysis

Ali F. Al-Hashimi, MSc, Faik H. Mohammed, PhD, Arif S. Al-Khazragi, CIBM.

ABSTRACT

Objective: To evaluate the role of oxidative stress (OS) in chronic renal failure (CRF) and the effect of peritoneal dialysis on the OS in uremic patients. Also, to investigate the role of the studied parameters of OS as early markers for the detection of peritonitis in peritoneally dialyzed patients.

Methods: The study was conducted on 80 chronic renal failure Iraqi patients who were admitted to the dialysis centers at Al-Kadhumiya, Baghdad, Al-Yarmouk and Al-Karama teaching hospitals, Baghdad, Iraq, during the period November 1999 through to July 2000 for peritoneal or hemodialysis therapy. Their ages range between 15-75-years. This was carried out by measuring the plasma values of malondialdehyde (MDA), thiol group, albumin, uric acid and total bilirubin before and after the dialysis session, compared to age and sex matched healthy controls.

Results: The significantly higher plasma MDA with lower plasma thiol levels prior to the dialysis session indicated most likely an increased OS in CRF patients, which has significantly decreased after the dialysis session. This OS was found to be significantly correlated with the degree of renal insufficiency measured by serum creatinine levels. In patients who developed peritonitis, post dialysis findings were in favor of an increase rather than a decrease in OS. Such findings were found prior to the clinical or biochemical diagnosis of peritonitis or both in most patients. Finally, in patients on regular hemodialysis therapy, results suggested a minor OS compared to patients admitted for peritoneal dialysis therapy.

Conclusion: Patients with CRF are subjected to an increased OS, the degree of which is related to the severity of renal failure. Moreover, plasma levels of the studied markers of OS do point in the direction of a decrease in the OS post dialysis. Such markers can be used for early detection of peritonitis in peritoneally dialyzed patients. Finally, chronic regular dialysis therapy is a more effective replacement therapy.


A free radical (FR) is a molecule or molecular fragment that contains one or more unpaired electrons in its outer orbital. The particular configuration of these radicals provides them with biologic properties leading to their intervention in various pathophysiologic situations. Reactive oxygen species (ROS) that are constantly formed within the body from oxidation-reduction reactions due to incomplete reduction of molecular oxygen, such as the superoxide anion radical (O2-), the hydrogen peroxide (H2O2) and the highly reactive hydroxyl radical (OH), are responsible for many of the biological effects of FRs within the human body. Oxidative stress results in chemical alterations of bio-molecules, causing structural and functional modifications.

Over the last decade, experimental evidence had accumulated indicating that the ROS play a key role in the pathophysiology of a wide variety of clinical and experimental renal diseases, making the kidney unique among other organs as the site in which a wide range of diseases involves the ROS.
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injury by ROS that can be generated from different sources within the kidney results from lipid peroxidation (LP) of mitochondrial, lysosomal and plasma membrane and perhaps by inactivation of sulfhydryl groups on functional proteins. The damaging effects of ROS can be controlled by various antioxidant defence systems such as superoxide dismutase (SOD) and glutathione peroxidase (GSHPxs) enzymes, ceruloplasmin, ascorbate, vitamin E, uric acid, albumin and bilirubin. Direct detection of FR in vivo has proved difficult by the routine techniques, as they tend to be extremely reactive and unstable. Nevertheless, identifying an increase in LP products or a disturbance in the antioxidant capacity or both have been used as an indirect evidence for the involvement of ROS in different diseases, and as a measurement of the degree of OS.

The study was conducted to evaluate the role of OS in CRF and to study the effect of peritoneal dialysis on the OS in uremic patients, through the measurements of plasma malondialdehyde (MDA-an LP product), plasma thiol, albumin, uric acid and bilirubin. In view of the observation of increased susceptibility to infection in peritoneal dialysis and the low sensitivity and specificity of the methods available for the diagnosis of peritonitis, we have attempted to investigate whether changes in the measured parameters can be used as early markers for the detection of peritonitis. The effects of peritoneal dialysis and hemodialysis on plasma MDA and thiol levels were compared.

Methods. The study was conducted on (80) patients suffering from CRF attending the dialysis centers at Al-Kadhumiya, Baghdad, Al-Yarmouk and Al-Karama teaching hospitals, Baghdad, Iraq, in the period between November 1999 through to July 2000. These were (32) females and (48) males, with an age distribution of 15-75 years. Mean age was (46.7 ± 15.4) years. The underlying renal diseases were: Hypertensive nephropathy (n=30), diabetic nephropathy (n=15), obstructive uropathy (n=11), chronic glomerulonephritis (n=4), chronic urinary tract infection (n=2), adult polycystic kidney disease (n=3) and unknown causes (n=15). Those patients were divided into 3 groups: Group I: Patients treated by peritoneal dialysis and ended their dialysis session without developing peritonitis (n=36). Group II: Patients developed peritonitis during or after their peritoneal dialysis session (n=24). Group III: Patients scheduled in the hemodialysis program and maintained on hemodialysis for 3 months up to 2 years (n=20). In the dialysis centers, 2 liters of dialysate solution kept inside the peritoneal cavity of CRF patients for 30 minutes and then replaced by a new dialysate solution. This process that continued for up to 72-hours, stopped when there was a clinical and a biochemical improvement. The peritoneal dialysis fluids contained sodium chloride (5.60g/L), calcium carbonate (0.26g/L), magnesium carbonate (0.15g/L), sodium lactate (5.0g/L), potassium chloride (0.3g/L), and glucose (13.6 and 70.0 g/L) with an osmolality ranges of 363-685 mOsm/L. Diagnosis of peritonitis was established when any 2 of the following criteria were present: Signs and symptoms of peritoneal inflammation (such as fever, abdominal pain and rebound tenderness), cloudy or turbid effluent containing >100 polymorphonuclear leukocytes (PMNL)/mm³ or a positive fluid culture. On the other hand, hemodialysis therapy consists of a 3- 4-hour session with 1-2 sessions per week. The dialyzer used is cuprophan dialyzer, Spiraflo-NT1175. The control group consisted of 20 non acutely infected subjects comparable in age and sex, with no history of renal diseases or any systemic illnesses except for hypertension and diabetes mellitus in some of them. All participants gave their informed consent to enter the study, which was approved by the ethical committee. The clinical characteristics of the studied subjects are presented in Table 1. From each patient, a (5 ml) blood sample was taken from the antecubital vein just before the beginning of the dialysis session (Peritoneal or hemodialysis). A second (5 ml) blood sample was taken just at the end of the dialysis session. For those patients who developed peritonitis, the second blood sample was collected before starting the antibiotic therapy. For control group, only one blood sample was collected. Blood samples were put into glass tubes with Ethylenediamine-tetra-acetic acid (EDTA) (1.5 mg/ml) as an anti-coagulant, and centrifuged at 2000 rpm for 15 minutes. the plasma was separated and stored at (-20°C) till the time of the biochemical analysis. The storage of the samples was not more than 2 weeks.

Biochemical assays. The plasma malondialdehyde (MDA) was measured in all samples according to Guidet and Shah method and expressed as nmol/ml. The plasma thiol concentration was measured according to Ellman method and expressed as μmol/L. Plasma albumin was measured by using available kits (Randox Laboratories Limited, Ardmore, Diamond Road, Crumlin, County Antrim, United Kingdom, BT29 4QY). The absorbance was read at 630 nm. Enzymatic determination of uric acid in the plasma was carried out by commercially available kits (Acide urique enzymatique PAP 150, Biomerieux, RCS Lyon B 673 620 399, 69 280 Marcy-l’Etoile, France). The absorbance was read at 520 nm. And finally, calorimetric method for determination of the total plasma bilirubin was performed through available kits (Randox Laboratories Ltd, Ardmore, Diamond Road, Crumlin, County Antrim, United Kingdom, BT29 4QY).

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Table 1 - The clinical characteristics of the studied groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>36</td>
<td>24</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Age/years (mean ± SD)</td>
<td>51.5 ± 17.14</td>
<td>48.5 ± 14.3</td>
<td>40.3 ± 14.2</td>
<td>43.56 ± 15.5</td>
</tr>
<tr>
<td>Gender</td>
<td>24:12</td>
<td>11:13</td>
<td>13:7</td>
<td>12.8</td>
</tr>
<tr>
<td>Blood urea (mg/dl)</td>
<td>183.4 ± 75.2</td>
<td>210.2 ± 45.6</td>
<td>111.67 ± 22.68</td>
<td>25.4 ± 6.1</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>9.12 ± 3.2</td>
<td>8.5 ± 3.8</td>
<td>5.87 ± 1.23</td>
<td>0.8 ± 0.3</td>
</tr>
</tbody>
</table>

* *p<0.0005 versus control group level
† p<0.0005 versus group 1 value

Table 2 - The changes of plasma malondialdehyde-LM and plasma thiol concentrations.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA Conc. (nmol/ml)</td>
<td>11.69*</td>
<td>10.45*</td>
<td>9.18*</td>
<td>8.59*++</td>
</tr>
<tr>
<td>Thiol Conc. (µmol/L)</td>
<td>929.7*</td>
<td>1012.46**+</td>
<td>896.17*+</td>
<td>1110.16++</td>
</tr>
</tbody>
</table>

MDA - malondialdehyde, Thiol - thiol group
* p<0.005 (versus control group)
** p<0.05 (control group)
+ p<0.005 (versus pre-dialysis value of the same group)
++ p<0.05 (versus pre-dialysis value of the same group)
§ p=0.003 (versus pre-dialysis value of group 1)

Table 3 - The changes of plasma albumin, uric acid and total bilirubin concentrations.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin Conc. (g/dL)</td>
<td>3.5</td>
<td>3.2*+</td>
<td>3.89</td>
<td>3.2*++</td>
</tr>
<tr>
<td>Uric acid conc. (mg/L)</td>
<td>86.99*</td>
<td>57.3+</td>
<td>88.93*</td>
<td>71.75**</td>
</tr>
<tr>
<td>Total bilirubin Conc. (mg/dl)</td>
<td>0.66</td>
<td>0.69</td>
<td>0.41</td>
<td>0.55</td>
</tr>
</tbody>
</table>

* p<0.005 (versus control group)
** p<0.05 (control group)
+ p<0.005 (versus pre-dialysis value)
++ p<0.05 (versus pre-dialysis value)

Figure 1 - Correlation between serum creatinine and plasma malondialdehyde concentrations
Kingdom, BT29 4QY), and the absorbance was read at 580 nm.

**Statistical analysis.** The results were expressed as (mean ± SD). Paired t-test was used to compare between the pre- and post-dialysis data for each of the studied parameters in each of the groups. Anova-test, and least significant difference-procedure were used to compare values of each of the studied parameters between the control and different patient groups and between the pre- or post-dialysis data of one group and its counterparts in the other groups. Bivariate correlation (Pearson) 2-tailed test was used to find the relation between the serum creatinine and plasma MDA concentrations in 40 uremic patients before starting peritoneal dialysis. The threshold of significance was chosen as \( p \leq 0.05 \) and the statistical package for social sciences package was used.

**Results.** As shown in Table 2, the pre-dialysis mean plasma MDA concentration of all patient groups was significantly higher than the control group level \((p < 0.005)\). The post-dialysis mean plasma MDA concentration had significantly decreased from the pre-dialysis level in group one \((p < 0.005)\) and group III \((p < 0.05)\) patients. However, it remained significantly higher than the value of the control group \((p < 0.005)\). Dialysis in patients of group II was associated with a significant increase in the mean plasma MDA as compared to the pre-dialysis value \((p < 0.005)\). In patients on regular hemodialysis therapy, both the pre and post-dialysis plasma MDA concentrations were lower than their corresponding value in group one, with a significant difference observed only in the pre-dialysis values \((p = 0.003)\). In the 3 groups, the pre-dialysis mean plasma thiol level was lower than that of the control group with a significant difference only in group one \((p < 0.005)\) and group 2 \((p < 0.05)\) (Table 2). Dialysis resulted in a significant increase in the mean plasma thiol, as compared to the pre-dialysis value in group one \((p < 0.005)\) and group 3 \((p < 0.05)\), whereas a significant decrease was observed in group 2 \((p < 0.005)\). However, the post-dialysis value remained significantly lower than control in group one \((p < 0.05)\). Finally, although the pre and post-dialysis plasma thiol levels in patients on hemodialysis were higher than their corresponding values in group one, the difference was not significant. In both groups one and 2, the mean plasma albumin before dialysis therapy was lower than the control group level, however, the difference was not significant. Peritoneal dialysis resulted in a significant decrease in plasma albumin concentration from its pre-dialysis levels and compared to controls in groups one and 2 \((p < 0.005)\) (Table 3). The pre-dialysis mean plasma uric acid (UA) concentration was significantly higher than control in patients of groups one and 2 \((p < 0.005)\). After peritoneal dialysis, there was a significant decrease in the plasma UA value in patients of group one compared to their pre-dialysis value \((p < 0.005)\). However, in patients of group 2, the post-dialysis decrease in plasma UA level was not significant and it remained significantly higher than the control group level \((p < 0.05)\). There was no significant difference between the control group value and the pre and post-dialysis plasma bilirubin concentrations of groups one and 2 (Table 3).

**Correlation between serum creatinine and plasma MDA.** In forty of the studied patients on peritoneal dialysis, Pearson correlation test shows a significant correlation between their serum creatinine concentrations and plasma MDA levels before starting peritoneal dialysis \((r = 0.667; p < 0.0005)\) (Figure 1).

**Discussion.** In the plasma of CRF patients, the levels of MDA, thiol, albumin, uric acid and bilirubin were compared with those of apparently healthy subjects. We found that the increase in plasma MDA associated with the decrease in plasma thiol in CRF patients suggests that there is an increased OS in CRF patients. Several studies have demonstrated the role of ROS in the pathogenesis of CRF, through the detection of increased products of LP process or disturbances of the antioxidant system (AOS) or both. The mechanisms whereby the ROS formation can cause glomerular morphological lesions and induce disturbances of the biomembrane morphofunctional states of the renal tubules, are inferred from in vitro studies. The nature of changes in plasma albumin, uric acid and bilirubin observed in our study were not conclusive in the evaluation of the role of OS in CRF, therefore, they were not compared with hemodialyzed patients (group 3). Human albumin antioxidant functions include the inhibition of copper-stimulated LP reactions and scavenging hypochlorous acid and peroxy radicals. It can also bind free fatty acids protecting them from peroxidation. In the present study, the low plasma albumin in CRF patients prior to peritoneal dialysis compared to the control group can be attributed to the state of protein intolerance that lead to malnutrition. The significant decrease in the post-dialysis plasma albumin concentration in groups I and II can be explained by loss of albumin into the peritoneal washout. Moreover, peritonitis add to the loss of albumin in patients of group II, as a consequence of increased capillary permeability mediated by chemicals secreted by the infiltrating neutrophils. Uric acid is generated in human body by the degradation of purines. It has been found that...
UA (or its sodium salt, urate) is a powerful antioxidant and is a scavenger of singlet oxygen and other radicals. In this study, the significantly higher pre-dialysis plasma UA concentration that we found compared to control, is a consequence of failure of the kidney excretory function as well as increased protein catabolism as part of the hypercatabolic state in CRF. Peritoneal dialysis, as a replacement therapy cause most of the metabolic end products to diffuse out into the peritoneal washout, which explains the significant decrease in the post-dialysis UA level in group one. While the non significant decrease in the post-dialysis UA level in patients who developed peritonitis, can be explained by the adding effect of infection and tissue damage to the hypercatabolic state in uremia. However, Hasegawa and Kuroda suggested that UA plays a role as an antioxidant in the plasma of CRF patients through detection of its oxidation product, allantoin, which was not found in normal plasma samples. Allantoin was also detected in other diseases with possible FR-reactions. Bilirubin, the end product of heme catabolism, is generally regarded as a potentially toxic compound when accumulated at abnormally high concentrations in tissues. However, it has been suggested that bilirubin acts as a powerful physiological chain-braking antioxidant that can efficiently scavenge peroxyl radicals, singlet oxygen and serves as a reducing substrate for peroxidases in the presence of hydrogen peroxide or organic hydroperoxides. Serum bilirubin contributes to around 10% of the total anti oxidant status of human blood.

In the current study, results of plasma bilirubin observed were not conclusive in demonstrating the possible role of OS in CRF. Shimoharada et al have demonstrated a marked increase in the concentrations of biopyrrins; novel oxidative metabolites of bilirubin in urine of oxidatively insulted patients, which was not correlated with the changes in serum bilirubin.

Changes in plasma malondialdehyde and plasma thiol concentrations. In the present study, the significantly elevated pre-dialysis plasma MDA concentration in patients of groups one and 2 compared to controls, which reflects an increased LP process was associated with a significantly lower plasma thiol concentration in the same patients, reflecting a lower antioxidant activity. From both, an increased oxidative stress (OS) in plasma of CRF patients can most likely be concluded. The increased OS, assessed by MDA levels in 40 CRF patients, was found to be correlated with the degree of renal dysfunction as measured by the serum creatinine concentrations prior to peritoneal dialysis (r=0.667; p<0.0005). These results are in agreement with the work of Fillit et al who found a significant TBA reactivity in blood of CRF patients compared to control blood which correlates directly with the degree of renal failure measured by serum creatinine concentration.

Peritoneal dialysis in patients of group I resulted in a significant decrease in plasma MDA level, and a significant increase in plasma thiol level compared to pre-dialysis data. The above finding would suggest that peritoneal dialysis most likely resulted in a decrease of OS in CRF patients who ended the peritoneal dialysis session without developing peritonitis. Peritoneal dialysis through the replacement of the renal excretory functions, will result in the removal of the various waste products with unknown uremic toxins that are possibly responsible for the generation of ROS in CRF patients. Several studies had demonstrated the presence of a plasma uremic toxin(s) or enhancing factor(s) that enhances the oxidative burst of PMNL in CRF patients. This toxin or enhancing factor was found to be specifically associated with renal dysfunction and dialyzable and could be useful for monitoring the progression of CRF.

In contrast, Fillit et al assumed the decrease in plasma TBA-reactive material, that this material is dialyzable itself. However, they reach to this conclusion without measuring the TBA-reactive material in the dialysate. A similar changes of plasma MDA and thiol group levels were observed in patients on regular hemodialysis therapy (group 3) following a hemodialysis session that suggest a decrease in the OS (as in group one). These results are in agreement with the study of Fillit et al. In addition, Takahashi and Imada found that hemodialysis had lowered the plasma-H2O2 production than that of the pre-dialysis in uremic patients. Compared with the results in group one, the significantly lower pre-dialysis plasma MDA and higher plasma thiol levels in patients of group 3 suggests that uremic patients on regular hemodialysis therapy have a minor OS that is also reflected on their better biochemical indices.
procedure has several advantages such as avoidance of vascular surgery and being more amenable to self-treatment (as in CAPD). However, several reports have introduced susceptibility to infection caused by deranged immunity, impaired inflammatory reactions and phagocytic function. Chronic renal failure patients suffer from increased susceptibility to infection caused by deranged immunity, impaired inflammatory reactions and phagocytic function. The reduced phagocytic and bactericidal ability in peritoneally dialyzed patients could be a consequence of an opsonic serum defect or a defect in neutrophils themselves, or both. Reduced activity of PMN intracellular AO enzymes or other factors related to PDF such as the low pH, high osmolality, large volume (2 liters) and the recurrent changing. The diagnosis and effective treatment of peritonitis depends on the correlation of clinical evaluation of the patient with laboratory examination of the patient. This raises the possibility that these parameters can be used as early markers for the detection of peritonitis during peritoneal dialysis procedure.

References

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