Salivary and plasma analysis of oxidative stress biomarkers in end stage renal failure patients

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ABSTRACT
Background: End Stage renal disease represents a clinical state or condition in which there has been an irreversible loss of endogenous renal function. To evaluate role of oxidative stress & anti oxidant in chronic renal failure patients by measuring malondialdehyde & uric acid in their plasma and saliva in comparison with apparently healthy control group.

Materials &methods: The study was conducted on 60 Chronic renal failure patients at Al-Hussein teaching hospital, Karbala (Iraq) & 30 apparently healthy control group. Laboratory measurements of salivary &plasma levels of oxidants ,antioxidants markers (MDA, uric acid) was done on hemodialysis patients compared to age & sex matched apparently healthy control group.

Results: Salivary & plasma (malondialdehyde , uric acid ) showed highly significant differences between chronic renal failure patients & apparently healthy control groups. This study was the first study that measure salivary MDA in end stage renal failure patients.

Conclusion: Higher salivary, plasma oxidants & antioxidants concentrations of end stage renal failure patients with increase salivary components disturbances in comparison with apparently healthy control group.

Key wards: Hemodialysis, Malondialdehyde, Uric acid. (J Bagh Coll Dentistry 2011;23(46-50)

INTRODUCTION
Chronic kidney disease (CKD) is a condition in which there is a functional loss of renal glomeruli caused by glomerular or interstitial renal disease. It is a worldwide public health problem (1).

Severe disturbance in the prooxidants-antioxidants balance in favor of the former, thus leading to a potential damage to the cells and organs(2).

Essentially, “oxidative stress” occurs when the formation of bioactive oxidation products greatly over whelms the capacity of endogenous cellular antioxidant defense system. Oxidative stress plays a role in a variety of renal diseases such as glomerulonephritis and tubulointerstitial nephritis, chronic renal failure, proteinuria, uremia(3). Patients on hemodialysis (HD) are constantly exposed to oxidative stress(4). This is mostly attributed to a bioincompatibility type of reactions, originating from the dialysis membrane and the imbalance between oxidants and antioxidants due to the diffusion of hydrophilic compounds to the dialysate. The intensity of oxidative stress in hemodialysis patients can be influenced by many factors among which are duration of dialysis therapy, primary cause of chronic renal failure (CRF), intensity of chronic inflammation, type of diet or environmental toxins(5). With the goal of developing a diagnostic test for the simultaneous detection of multiple markers in saliva, the selected end-stage renal disease (ESRD) as a suitable target disease state, because the disorder is a well-defined phenotype and its effect on blood composition is well known. Owing to the contribution of serum-derived components to whole saliva, we hypothesized that changes in serum composition caused by hemodialysis would be reflected in saliva (6).

MATERIALS AND METHODS
The subjects of this study were 60 individuals undergoing HD with chronic renal failure disease. A thirty two males (53.3%) with age range (25-63ys) and 28 females (46.7%) with age range(27-64ys). The hemodialysis duration (HD) range (4-60) weeks , All the patients were undergoing 3 hr of HD thrice a week. HD was prescribed in these patients with single-use hollow-fiber dialyzers equipped with modified cellulose-based or polysulfone membranes. The dialysate used was a standard ionic composition and bicarbonate-based buffer in all cases.

Blood samples
About 8mls of venous blood sample were aspirated from anticubital vein of each individual, using plastic disposable syringes with 23 gauge stainless steel needle. The whole blood was collected in sterile polyethylene tubes. plasma was obtained by centrifugation at 4000 rpm for 15 minutes, transferred immediately into another

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tube & frozen at (-20°C) for subsequent analysis. Haemolysed sample discarded.

**Saliva samples**

The patients and control group were instructed not to eat or drink (except water) for at least (1) hour before collection of the samples. Stimulated whole saliva was collected by chewing a piece of paraffin (10x10cm, and weighting 1.40 g). During the period of collection the individuals were comfortably seated in a ventilated and lighted room. Saliva produced in the first 30 seconds was discarded, then saliva was collected for exactly (5 minutes) in a graduated collection polyethylene tubes, a standardized method according to Thylstrup and FejersKov(7).

**Determination of oxidant & antioxidant markers**

1- (Malondialdehyde) MDA Estimation (Shah & Walker) (8).

Lipid peroxidation end products, particularly malondialdehyde (MDA) react with thiobarbaturic acid under acidic conditions and heated to give a pink color that measured spectrophotometrically at 532nm.

2- Uricase acts on uric acid to produce allantoin, carbon dioxide and hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase reacts with a chromogen (amino-antipyrine and dichlorohydroxybenzen sulfonate) to yield quinoneimine, a red color complex, the absorbance measured at 520 nm (490-530) is proportional to the amount of uric acid in the specimen.

**RESULTS**

Oxidative Stress Assessments Malondialdehyde (MDA).

**Plasma Malondialdehyde levels**

The presented results of the lipid peroxidation assessed by TBARS measurements (MDA) are shown in Table 1 & figure 1.

Plasma (MDA) levels were significantly higher in HD patients compared to the apparently healthy subjects, (MDA) levels of the study group (Mean ± SD) = (1.63±0.19), (p =0.016); MDA level of control group (1.52±0.22).

**Salivary malondialdehyde levels**

Salivary (MDA) levels were significantly higher in HD patients when compared to the apparently healthy subjects. MDA levels of the study group (Mean ± SD) = (3.33±0.37), MDA level of control group (2.94±0.44), (p < 0.0001), as shown in table (2) & figure (2).

Antioxidant Stress Assessments (uric acid):-

**Plasma & salivary Uric acid levels:-**

The mean & SD (Standard Deviation) of plasma & salivary uric acid mean showed highly significant differences increased changes in comparison to apparently healthy control group, as shown in figure 1, 2 & table 3,4: plasma & salivary uric acid investigations in chronic renal failure patients & control subjects.

<table>
<thead>
<tr>
<th>Table 1: Plasma MDA in study &amp; control group.</th>
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<tbody>
<tr>
<td>Plasma MDA No.</td>
</tr>
<tr>
<td>Patient 60</td>
</tr>
<tr>
<td>Control 30</td>
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<table>
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<th>Table 2: Salivary MDA in study &amp; control group.</th>
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<tr>
<td>Salivary MDA No.</td>
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<tr>
<td>Patient 60</td>
</tr>
<tr>
<td>Control 30</td>
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</tbody>
</table>

![Figure 1: Plasma MDA & uric acid in study & control group.](image1)

![Figure 2: Salivary MDA & uric acid in study & control group.](image2)
Table 3: Plasma uric acid in study & control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Mean± SD (Range)</th>
<th>SE</th>
<th>P</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>60</td>
<td>5.46± 0.66 (4.3-6.7) mg/dL</td>
<td>0.08</td>
<td>&lt;0.001</td>
<td>0.84</td>
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<tr>
<td>Controls</td>
<td>30</td>
<td>4.39± 1.27 (2.5-9) mg/dL</td>
<td>0.23</td>
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Table 4: Salivary uric acid in study & control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Mean± SD (Range)</th>
<th>SE</th>
<th>P</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>60</td>
<td>2.74± 0.86 (1.5-4.5) mg/dL</td>
<td>0.11</td>
<td>&lt;0.001</td>
<td>0.84</td>
</tr>
<tr>
<td>Controls</td>
<td>30</td>
<td>2.08± 0.64 (1.1-3.2) mg/dL</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Oxidative stress is suggested to play a role in hemodialysis, which increases the risk of cardiovascular diseases in patients on chronic dialysis. Enhanced OS in hemodialytic patients is a risk factor for accelerated atherosclerosis (9).

Oxidative stress marker malondialdehyde (MDA)

Plasma malondialdehyde (MDA).

Several studies have demonstrated increased oxidative stress in patients with CRF, including accumulation of reactive carbonyl compounds as markers of elevated protein peroxidation (10), increased concentrations of malondialdehyde in plasma as marker of high lipid peroxidation (11).

One reason for OS in patients with renal failure is the underlying disease itself. Renal toxicity and immunological disorders of the kidney result in an elevated formation of reactive oxygen species (ROS) which is active in the pathogenesis of kidney disease. However, treatment procedures were also shown to induce OS. Oxidative stress is particularly detrimental in patients receiving hemodialysis (HD) because there is massive and repeated at each dialysis session due to the contact of blood with dialysis membranes, facing to a chronic deficit in antioxidant defense system. Moreover, several lines of evidence have indicated that oxidative metabolism in peripheral and peritoneal phagocytes is activated during peritoneal dialysis (PD) with conventional dialysate characterized by high concentration of glucose, by glucose degradation products (GDP), and by low pH and high osmolality (12).

The presented results show significantly elevated level of plasma MDA highly significant differences were compared with those of apparently healthy subjects. These results suggest that there is an increased OS in CRF patients. This study in agreement with Mehri Kadkhodaei & Elshamaa (13,14). This study showed an inverse correlation between MDA serum concentration and hemoglobin in the blood of HD patients. This is in agreement with Lucchi & Elshamaa (10,14).

The accelerated LPO at the low Hb level 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 anaerobic metabolism due to hypoxemia and ischemia (15). 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 the polymorphonuclear lymphocyte and partial uncoupling of oxidative phosphorylation (16,17).

Salivary MDA

In the present study increase salivary MDA level as compared to apparently healthy controls shows highly significant differences. These results showed positive correlations between Plasma & Salivary MDA, Pearson’s correlation (R) = 0.858 which reflect direct effect of salivary MDA level by systemic oxidative stress. Ben-zevi showed increase oxidative stress in saliva & serum of CRF & hemodialised patients (6).

Antioxidant marker Uric acid Plasma uric acid

In this study, elevated level of plasma uric acid were significantly higher in chronic renal failure patients compared to those apparently healthy control subjects. These results suggests that there is an increased OS in CRF patients. The nature of changes in plasma uric acid is generated in human body by the degradation of purines. Recent studies in humans also suggest that uric acid is a true risk factor for kidney disease. Numerous recent papers have reported elevated uric acid as an independent risk factor for kidney disease in the general population (18,19).

Elevated uric acid has also been reported to be more common in patients with diabetes with progressive renal disease (20). While earlier studies have reported mixed results from lowering uric acid in patients with renal disease (reviewed by Johnson (21)), a recent clinical study found that lowering uric acid in patients with renal disease and asymptomatic hyperuricemia resulted in less progression of their renal disease (22). While these findings need to be confirmed, these studies, as well as reports by others, suggest that lowering uric acid may be another way to help slow the progression of renal disease (23).
Salivary uric acid

The hypothesis that salivary UA is a suitable marker for qualitatively evaluating dialysis efficacy, given that patients experiencing renal failure have high plasma and salivary UA concentrations (24).

In the present study increase salivary uric acid level as compared to apparently healthy controls which shows highly significant differences & positive correlation of UA in plasma and saliva between patients and apparently healthy control group, these results in agreement with Bibi & Timothy (25,26).

Goll and Mookerjee also observed that UA and creatinine concentrations in whole saliva correlated significantly with those in serum and speculated that a saliva diagnostic test would be useful for reducing the number of blood tests for anemic and pediatric patients with renal disease. The results we have presented support the notion that changes in UA concentrations occurring during dialysis can be monitored in saliva; therefore salivary UA warrants further examination for its clinical utility (27).

The body containing a number of protective antioxidant mechanisms, whose specific role is to remove harmful oxidants as they form, or to repair damage caused by reactive oxygen species. Recent medical and dental research is geared towards prevention of free radical medicated diseases by using specific antioxidants. Preliminary data indicates protective role of antioxidant supplementation in prevention of precancerous lesions and periodontal diseases (28,29).

REFERENCES

22. Siu YP, Leung KT, Tong MK, Kwan TH. Use of allopurinol in slowing the progression of renal disease.