Enzymatic Study in Sera of Chronic Renal Failure Patients Undergoing Hemodialysis

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Abstract
This study involved an estimation of the activity of some enzymes such as xanthine oxidase (X.O), acidic and alkaline deoxyribonuclease (DNase), peroxidase and pepsinogen in sera of patients with chronic renal failure undergoing hemodialysis. The study included 32 patients with chronic renal failure of both sexes, compared with 63 controls of health as a control group. The results showed a significant increase in the activity of xanthine oxidase (X.O), acidic and alkaline deoxyribonuclease (DNase) and pepsinogen enzymes before and after hemodialysis, compared with the control group for both sexes, while a significant decrease was observed in the activity of peroxidase (P<0.001) enzymes, which showed a significant correlation between the level of the enzymes in the sera of patients before and after hemodialysis.
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failure (CRF) which treated with hemodialysis. Blood samples of 32 patients with chronic renal failure from both sexes were compared with (63) samples taken from healthy individuals as a control group.

The results showed a significant increase in the activity of xanthine oxidase, acidic and alkaline DNase and pepsinogen enzyme in serum of patients with CRF before and after hemodialysis compared with control for both sexes. A significant decrease was noted in activity of peroxidase in sera of patients with CRF before and after hemodialysis compared to control group for both sexes.

The results also demonstrated that the hemodialysis process caused a significant reduction (P≤0.001) in the activity of xanthinoxidase and pepsinogen. No significant effect (P>0.05) for hemodialysis on the reduction of acid DNase and elevation of alkaline DNase levels was noted. Hemodialysis had a significant increase (P≤0.001) on peroxidae activity.

Furthermore, correlation coefficient between the levels of enzymes in sera of patients before and after hemodialysis were determined.

Introduction

Chronic renal failure (CRF) is the stage in chronic renal disease when renal dysfunction has progressed to a level resulting in systemic manifestations. These manifestations include a rise in the blood concentration of urea, creatinine, which are removed by the kidneys, and other problems, such as anemia, bone disease, acidosis, and salt and fluid retention\[1]. CRF is a worldwide public health problem with an increasing incidence and prevalence associated with poor outcomes, and high cost\[2,3]. CRF is a slowly progressive loss of renal function over a period of months or years and an abnormal low glomerular filtration rate, which is usually determined indirectly by the creatinine level in blood serum\[4]. Waste products and excess water accumulate throughout the body, causes of renal failure include vascular problems as well as trauma, infection, or exposure to chemicals or medications. The kidney cannot filter the waste and water adequately in any of these stages, but the severity of the condition varies widely. End stage renal failure (ESRD) is the last stage of chronic renal failure and often requires dialysis or kidney transplantation as life saving measures\[5].

Uremia is a presence of excessive amounts of urea and waste products in the blood, which may be produced sign of kidney diseases or failure\[6]. Uremia is a toxic condition resulting from renal failure, when kidney function is compromised and urea, a waste product normally excreted in the urine, is retained in the blood. Uremia can lead to disturbances in the platelets and hypersomnia, among other effects\[7].
CRF that leads to severe illness and requires some form of renal replacement therapy (such as dialysis) is called end-stage renal disease (ESRD).\[4\]

Dialysis means a procedure that performs many of the normal duties of the kidneys, like filtering waste products from the blood, when the kidneys no longer work adequately. There are two types of dialysis: hemodialysis and peritoneal dialysis\[8\]. Dialysis process by which biologic waste products are removed from the body through external blood circuit and external (artificial) membranes\[1\]. Daily hemodialysis or continuous hemofiltration may be required initially to remove urea and potassium that are released from damaged muscles. This allows gradual removal of solutes and the slow correction of fluid overload\[9\].

**Aim of study:**

The aim of this study was to study the effect of continuous dialysis attendance on the enzymes level, changes in enzymes activity and its consequent effects, also to determination the enzymes activity in the serum of chronic renal failure patients before and after hemodialysis to clarify the effect of this disease on the level of these enzymes.

**Materials and Methods**

The patients of chronic renal failure treated by hemodialysis as they were submitted to the artificial kidney unit at Ibn-sena and azade training hospital in Nineveh and Duhok provinces/North of Iraq were selected for this study. Different individuals were selected as control healthy groups.

Venous blood samples (10) ml was drawn from (32) patients of chronic renal failure ranging between (15-70) years old. Samples, then transferred immediately to a clean dry plain tube. After removing the needle, the blood was allowed to clot for at least 10-15 min. at room temperature, centrifuged for (10) min. at (4000xg). Serum was removed for the measurement of biochemical parameters\[10\]. Blood serum was obtained from (63) healthy individuals ranging in age between (15–65) years as a control groups were selected from Duhok University students and their civilians as a control groups.

All biochemical parameters in blood serum were determined by using spectrophotometer, Pepsinogen activity was estimated according to\[11\], peroxidase activity was determined according to the\[12\]. Xanthine oxidase activity was estimated according to\[13\], also acidic and alkaline DNase activity was measured by the modified method of\[14\].

**Statistical analysis:**

The results were statistically analyzed by using t-test by comparing biochemical factors between control group and patient group. The results
were expressed as mean ±SD. Duncan’s test was used to differentiate between the mean values for blood biochemical parameters. Analysis of variance (ANOVA) was used to find the significant between groups. The comparison included CRF patients and healthy control groups. The means were distinguished among statistical groups at P < 0.05, has been taken as statistically significant\textsuperscript{[15]}.

**Results and Discussion**

The result are summarized in tables (1, 2 and 3). The xanthinoxidase, acid and alkaline DNase and pepsinogen activity showed a significant increase (P<0.05) in serum of CRF patients before hemodialysis compared to control group. Also, a significant reduction (P<0.05) in peroxidase levels was noticed in serum of CRF patients before hemodialysis.

Table (1): Effect of dialysis on the level of some biochemical parameters in patients of chronic renal failure.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>No.</th>
<th>Mean±SD</th>
<th>p*-value</th>
<th>Duncan's Grouping**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Xanthine oxidase(U/L)</td>
<td>Before hemodialysis</td>
<td>32</td>
<td>13.6±1.7</td>
<td>&lt;0.001*</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>After hemodialysis</td>
<td>32</td>
<td>11.3±1.9</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>63</td>
<td>8.3±2.2</td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>2-Acid DNase (U/L)</td>
<td>Before hemodialysis</td>
<td>32</td>
<td>32.0±5.0</td>
<td>&lt;0.001*</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>After hemodialysis</td>
<td>32</td>
<td>30.8±4.0</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>63</td>
<td>16.0±2.2</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>3-Alkaline DNase(U/L)</td>
<td>Before hemodialysis</td>
<td>32</td>
<td>8.9±1.6</td>
<td>&lt;0.001*</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>After hemodialysis</td>
<td>32</td>
<td>9.2±1.7</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>63</td>
<td>4.0±1.1</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>4-epsinogen (μmol/ml)</td>
<td>Before hemodialysis</td>
<td>32</td>
<td>42.5±5.3</td>
<td>&lt;0.001*</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>After hemodialysis</td>
<td>32</td>
<td>34.1±3.8</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>63</td>
<td>30.4±3.3</td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>5-Peroxidase (U/L)</td>
<td>Before hemodialysis</td>
<td>32</td>
<td>34.1±4.17</td>
<td>&lt;0.001*</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>After hemodialysis</td>
<td>32</td>
<td>37.8±2.91</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>63</td>
<td>46.0±0.39</td>
<td></td>
<td>C</td>
</tr>
</tbody>
</table>

* Significant difference at (p ≤ 0.05).
** Means with different letters were statistically significant at (p ≤ 0.05).

1- Xanthine Oxidase activity (X.O):
The results in Table (1) showed a significant increase (P<0.05) in xanthine oxidase activity in CRF patients before hemodyalysis compared to healthy control group. The higher activity of X.O may be due to the function of the enzyme which catalyzes the breakdown of nucleotides to form uric acid. Uric acid contributes to the antioxidant capacity of the blood. This enzyme is involved in the pathogenesis of tissue injury by the production of superoxide anion radicals and hydrogen peroxide or
both\textsuperscript{[16,17]}. Or may be indicating that plasma X.O is not transferred to the urine, probably due to its high molecular weight\textsuperscript{[18]}.

The studies by Borges et al., 2002, had used xanthine oxidase inhibitors as a means to prevent (CRF) in animals, also showed that the X.O levels in blood had significantly increased in different pathological cases, like aging, ischemia reperfusion, inflammation and cancer, and that reactive oxygen species generated in the enzymatic process were involved in oxidative damage\textsuperscript{[19]}. The reason for increasing of xanthine oxidase activity in blood serum may due to the rises in DNase activity in(CRF) patients because the rises in DNase activity lead to breakdown of DNA to small nucleotides so, the xanthine oxidase activity must increase to convert these nucleotides to uric acid\textsuperscript{[17]}.

Statistical analysis Table(1) showed a significant increase (P<0.05) in X.O levels, in serum of CRF patients after hemodyalysis in comparison to control group for both sexes, also the statistical analysis showed a significant reduction (P<0.05) in X.O activity in serum of CRF patients after hemodyalysis when compared to the activity before dialysis for both sexes.

2- Deoxyribonucleases activity (DNase):

Table (1) showed a significant increase in the activity of acid DNase in(CRF) patients before hemodyalysis (P<0.05) compared to control groups. The increase of acid DNase activity might be due to that the DNase plays important roles in DNA fragmentation and degradation during programmed cell death. \textsuperscript{[20]},or the increase might be due to a sign tissue damage which lead to the changes in cell membrane permeability and the enzyme released in to the serum\textsuperscript{[21]}. Statistical analysis showed a significant elevation (P<0.05) in acid DNase activity in CRF patients before and after hemodyalysis compared to control groups.

Results in Table (1) showed a significant increase (P<0.05) in alkaline DNase activity in serum of CRF patients before hemodyalysis compared to control group, the reason for that due to the disease process may increase cell death and resulting in release of intracellular enzyme in to the plasma\textsuperscript{[21]}. No significant differences (P>0.05) in the (DNase) activity before and after hemodyalysis for both sexes.

3- Pepsinogen (PG):

Pepsinogen, the precursors of pepsin, in patients with impaired renal function are associated with elevated concentrations of serum pepsinogen. Previous investigations reported that patients undergoing dialysis therapy had significantly higher serum pepsinogen (PG) levels than those with normal renal function\textsuperscript{[22,23]}. Paimela et al.,1985 found, that the dialysis patients infected with Helicobacter pylori was eradicated showed a significant reduction of serum PG levels \textsuperscript{[24]}. 
The activity of pepsinogen in serum of CRF patients before hemodialysis was significantly increased (P<0.05) when compared to control group. The reason for increasing of pepsinogen in CRF patients might be due to the effect of *H. pylori* in dialysis patients because of a significantly correlated with the inflammation, mucosal inflammation and activity scores of antrum in dialysis patients, and these scores were highly influenced by *H.pylori* infection\[^{23,24}\]. Or may be due to the hyper secretion of gastrin in serum because the levels of gastrine were higher in dialysis patients than in controls and the mechanism of increased gastrin concentration in *H. pylori* infection, is that *H. pylori* produces a specific biochemical substance that stimulates G-cell function or that inflammation self stimulates gastrin hypersecretion\[^{22}\].

Kotanko *et al.*, 2006, suggested that the intestinal bacteria contribute to the uraemic syndrome by the production of uraemic toxins and the translocation of bacteria from the gut to the blood takes place in kidney failure. Consequently, it is assumed that the gut contributes to the chronic inflammatory state in dialysis patients \[^{25}\]. Statistical analysis Table (1) showed a significant increase (P<0.05) in pepsinogen activity in serum of CRF patients after hemodyalysis in comparison to control group for both sexes and a significant decrease (P<0.05) in pepsinogen in serum of patients with CRF after hemodyalysis compared to patient before hemodyalysis for both sexes. The obtained results were in agreement with those reported by Paimela *et al.*, 1985, which showed a lower pepsinogen levels in CRF patients after dialysis \[^{24}\].

4- Peroxidase:

The results in Table (1) showed that there was a significant decrease (p<0.05) in peroxidase activity in serum of CRF patients before hemodyalysis compared to the control group. These results are in agreement with those obtained by Díez, 2003 \[^{26}\]. The reduction of serum peroxidase activity on patients before hemodyalysis might be due to that the kidney is the main source for the plasma peroxidases and the its damage lead to elevation of reactive oxygen species and oxidative stress in blood, therefore the preventive antioxidants like peroxidase may be used for scavenging reactive oxygen species and the peroxidase enzyme decreased\[^{27}\].

It was noted\[^{28}\], that the low peroxidase activity in serum of CRF patients may be due to that the enzyme act as preventive antioxidants to detoxify damaging lipid peroxides or other peroxides from blood and organic substrates and can also modify low density lipoprotein (LDL) in the presence of H\(_2\)O\(_2\) or lipid hydroperoxide (LOOH) (scavenge lipid peroxide). Other explanations for the decrease this enzyme activity might be due to that the kidney damage occurs through a number of biochemical
mechanisms, all of which have in common the formation of highly reactive free radicals that can oxidize proteins, lipids and nucleic acids\(^{[29]}\), and increase the peroxide formation in the kidney\(^{[30]}\).

The results in Table (1) showed that there was a significant decrease (\(p<0.05\)) in peroxidase activity in serum of CRF patients after hemodialysis compared to the control group. These may be due to that the activities of peroxidase enzyme will be changed in hemodialysis patients due to the dialysis process, since it enhance the production of free radicals and reactive oxygen species because of inefficiency of the used dialysis membrane because it is saturated with various bacteria products able to cross the membrane via the Dialysate to blood components\(^{[31]}\).

Also the statistical analysis showed that the hemodialysis process increased peroxidase activity in serum of CRF patients after hemodialysis when compared to the activity before dialysis for both sexes. These results disagree with those obtained by Kaysen ,2001, which showed that the dialysis process did not reduce oxidative injury and may actually exacerbated oxidative stress\(^{[31]}\).

### Relationship among measured biochemical parameters in sera of chronic renal failure patients undergoing hemodialysis:

To find a relation between the biochemical parameters in sera of chronic renal failure patients undergoing hemodialysis, the correlation coefficient "\(r\)" was found. Table (2) proved to show a direct significant relationship between the level of Xanthine oxidase and pepsinogen before dialysis \(p \leq 0.05\), \(r=0.413\). Table (3) show a direct significant relationship between the level of Xanthine oxidase and alkaline DNAse after dialysis \((p \leq 0.05\), \(r=0.403\)).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pepsinogen</th>
<th>Acid DNAse</th>
<th>Alkaline DNAse</th>
<th>Xanthine oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid DNAse</td>
<td>Correlation</td>
<td>-0.035</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline DNAse</td>
<td>Correlation</td>
<td>0.104</td>
<td>-0.102</td>
<td></td>
</tr>
<tr>
<td>Xanthine Oxidase</td>
<td>Correlation</td>
<td>0.413*</td>
<td>0.082</td>
<td>0.252</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>Correlation</td>
<td>-0.074</td>
<td>0.202</td>
<td>-0.120</td>
</tr>
</tbody>
</table>

* Correlation is significant at level \((p \leq 0.05)\).  
** Correlation is significant at level \((p \leq 0.01)\).
Table (3): Correlation coefficient "r" for CRF patients after hemodialysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pepsinogen</th>
<th>Acid DNase</th>
<th>Alkaline DNase</th>
<th>Xanthine oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid DNase</td>
<td>Correlation</td>
<td>–0.084</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline DNase</td>
<td>Correlation</td>
<td>0.089</td>
<td>0.149</td>
<td></td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>Correlation</td>
<td>0.089</td>
<td>–0.110</td>
<td>0.403*</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>Correlation</td>
<td>0.217</td>
<td>0.036</td>
<td>0.153</td>
</tr>
</tbody>
</table>

* Correlation is significant at level ($p \leq 0.05$).

Conclusions:
The increase in PG activity in CRF patients might be considered as a biochemical marker for kidney functions associated with renal failure. and the determination of pepsinogen activity which was not measured before that for patients at dialysis centre (in artificial kidney unit at Ibn-sena and azade training hospital in Nineveh and Duhok provinces) has an important role in studying the effect of continuous dialysis attendance on the enzyme level and its consequent effects.

From the foregoing results, it could be concluded that the oxidative stress, can be used to help the diagnoses and identify the severity of process and studied determine the stage of kidney diseases. Also the levels of the enzymes might be of a great help to manage of such patients, since these enzymes can be standardized and used as a usual investigations. The increase in (X.O, acid and alkaline DNase and pepsinogen) activities could be as a good marker for CRF. Finally, the decrease in the peroxidase activity which was noted in the case of renal failure can de used as a sign for the diseases.

References
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