Study of some biochemical and hematological parameters in continuously hemodialysis patients in Kirkuk city

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Abstract

Introduction: The severity of chronic renal failure (CRF) classified by professional guidelines in five stages, stage 5 is a severe illness often called end stage kidney failure or end stage renal disease (ESRD), dialysis may be used for this stage. Dialysis treatments replace some of these functions through diffusion (waste removal) and ultrafiltration (fluid removal). Hemodialysis, is a process of purifying the blood, when the kidneys are not working normally. It is one of three renal replacement therapies (the other two being peritoneal dialysis and renal transplant).

Materials and Methods: The samples of this study consists of 80 patients (42 males, 38 females) and 85 healthy individuals (43 males, 42 females) from (20 - 70) years old of age. Serum paraoxonase-1 (PON1), 5-nucleotidase, blood urea (BU), serum creatinine (Cr), serum iron (Fe), serum total iron binding capacity (TIBC), Serum ferritin, serum amylase activity and serum lipase activity were determined. Some hematological parameters includes packed cell volume (PCV), hemoglobin (Hb), white blood cells count (WBCs), red blood cell count (RBCs) were measured, the erythrocyte sedimentation rate (ESR), Body mass index (BMI) and creatinine clearance (Ccr) were also measured.

Results: Decreased levels of some enzymes activity and some biochemical and hematological parameters and increased others in continuous and regular hemodialysis patients in Kirkuk city. Blood urea and serum creatinine showed significant increased (P<0.05) in their levels, while the creatinine clearance was significantly decreased (P<0.05) in patients with continuous hemodialysis patients when compared to those of controls. In the present study; there was significant decreased (P<0.05) in PON-1 in patients when compared to those of controls. However, the serum’s 5-NT, amylase and lipase activities were significantly increased (P<0.05) in these patients when compared with serum of normal individuals. In the present study; there was significant decreased in Fe and TIBC concentrations in the serum of patients when compared to those of healthy control individuals (P<0.05). While the serum ferritin was significantly increased (P<0.05) in these patients as compared to the controls. In the present study; there was significant decreased (P<0.05) in Hb and PCV and RBCs were measured, the erythrocyte sedimentation rate (ESR), Body mass index (BMI) and creatinine clearance (Ccr) were also measured.

Conclusion: Serum paraoxonase PON-1 activity is the most commonly affected than other biochemical parameters in continuous hemodialysis patients. Anemia, pancreatitis are the common and often an early complication of chronic renal diseases.
1- Introduction

One of the major public health problems is chronic renal failure (CRF)[1], its prevalence still world wide growing[2]. CRF prompts a slow and gradual decline of kidney function. It is usually caused by complications of other diseases. Unlike the acute renal failure (ARF), which happens sharply and suddenly, CRF happens gradually - over a period of weeks, months, or years[3]. If the kidneys are functioning at less than 10% of their normal capacity, i.e., the glomerular filtration rate (GFR) is lower than 15 ml/ min/ 1.73 m² and the chronic renal failure reaches the end-stage renal disease [ESRD][4].

Uremic syndrome is the clinical signs and symptoms of ESRD, hypertension, diabetes and other metabolic and physiologic derangements are the complications of ESRD[5]. Dialysis used in this stage[6] for removing toxic end products of metabolism such as urea and excess fluids from the plasma by dialyzing the blood against fluid containing no urea[7]. Paraoxonase (PON-1) is an enzyme associated with high density lipoprotein (HDL) and synthesized in the liver mainly, its substrates are unknown but recent studies suggest that the oxidation of low density lipoprotein (LDL) prevented by paraoxonase by hydrolyzing lipid peroxides[8]. The active site of PON-1 includes free thiol (-SH) group present in cysteine - 283 which donates reducing equivalent to PON-1 giving to it a reducing feature[9]. HDL associated glycoprotein PON-1 facilitates some of its systemic antioxidant protection; protection against lipoprotein oxidation and oxidized phospholipids remodeling[10]. Calcium-dependent esterase PON-1 hydrolyzes lipid peroxides, including paraoxon. PON-1 is a glycoprotein with 43-45 KD molecular weight which has a six - bladed β - propeller shape and two calcium ions located in the central tunnel of the enzyme that are important for both catalytic function and structure stability[11].

PON-1 activity has been restored by renal transplantation and it is better activated by salt than that of control subjects, causing qualitative changes in the molecule[12]. Several groups in numerous studies confirm these findings[13]. The level of Paraoxonase activity may be affected by non-genetic factors such as diet, smoking and hormonal status[14]. Furthermore, serum paraoxonase activity was found to be reduced in a number of pathological conditions including myocardial infarction, diabetes and hypercholesterolemia[15]. Many studies have demonstrated that PON-1 serum activity was also decreased in continuously hemodialysis patients[16]. The enzyme '5'-nucleotidase in mammalian cells is mainly located in the plasma membrane and its primary action is in the conversion of extracellular nucleotides (e.g.5'-AMP), which are generally impermeable, to the analogous nucleoside (e.g. adenosine) which can easily enter most cells. Consequently, '5'-nucleotidase plays a key role in the metabolism of nucleotides[17]. In addition to that, the concentrations of the serum of two pancreatic enzymes; amylase and lipase are increased in ESRD patients under hemodialysis and without of acute pancreatitis. The increased degree is nearly commensurate to the degree of renal failure[18]. The level of serum amylase and amylase clearance were scrutinized in patients of CRF without pancreatitis. The clearance of amylase was significantly lower in patients with strongly impaired renal function, and the level of serum amylase was markedly increased because of decreasing the excretion of the enzyme, but the increases were not higher than that of pancreatitis[19]. Anemia usually happens in patients with the continuous, partial loss renal function in CRF disease and it gradually develops from first to advanced stages of the CRF. Nearly all the patients who have kidney failure or sever impaired of kidney function, have anemia[20]. The type of anemia that is more common in chronic renal failure especially in continuously hemodialysis patients is iron deficiency anemia. It depends on a number of factors or causes, includes elevation of inflammation, lowering of erythropoietin EPO production, and increased of uric acid concentration level leading to bone marrow repression and decreased erythrocyte production[21]. Anemia is a big problem in patients with (CRF) disease on continuous hemodialysis, it could be diagnosed as normochromic, normocytic anemia with no leukopenia or thrombocytopenia[22]. This is a continual complication which has a serious role in reducing the quality of life[23].of CRF patients causing elevation of the rate of morbidity and death[24]. The levels of serum ferritin are estimated in continuous hemodialysis patients as part of serum iron studies in anemia. When the serum ferritin levels increased, the level of iron will be in excess, this mean that there is a direct relation with total quantity of stored iron in the body including cases of anemia of chronic renal failure[25].

2- Materials and Methods

The present study was conducted from June 2014 t0 September 2015 on patients attending the Kidney Dialysis Unit at Kirkuk General Hospital. The samples consist of 80 patients (42 males, 38 females) and 85 apparently healthy individuals (43 males, 42 females) from (20 - 70) years old of age. All patients included in this study were non diabetic, non alcoholism, without viral hepatitis and undergoing continuously regular hemodialysis therapy. Serum paraoxonase-1(PON1) activity has been measured spectrophotometrically [26]. The '5'-NT activity was measured by following Wood and Williams method[27] blood urea (BU) was measured modified urease-berthelot method [28].Serum creatinine (Cr) was estimated by kinetic test without deproteinization [29], serum iron (Fe) was determined by colorimetric method[30], total iron binding capacity (TIBC) were measured also by using colorimetric methods[31], ferritin level was determined by Enzyme Linked
Florescent Assay (ELFA)\(^2\). Serum amylase activity was measured using Wootten method\(^3\). Serum lipase activity was determined with a conventional turbidimetric assay without co – lipase[34]. The hematological parameters: hemoglobin (Hb) concentration, packed cell volume (PCV), total white blood cells count (WBCs), red blood cell count (RBCs) and erythrocyte sedimentation rate (ESR) were measured by Coulter Counter (Sysmex K-1000), TOA medical electronics CO., LTD. KOBE. JAPAN [35], determination of Body Mass Index (BMI) was estimated by the equation: BMI = weight (kg) / height (m\(^2\))[36]. The value of creatinine clearance (CrCl) was evaluated from the creatinine concentration in the collected urine sample (Ucr), volume of the collected urine (usually for 24hour) and the plasma concentration (Pcr) by the following equation[37]:

\[
\text{CrCl} = \frac{\text{UCr} \times \text{24-hour volume}}{\text{Pcr} \times 24 \text{ hour} \times 60 \text{ min}}
\]

3- Statistical analysis

The statistical analysis of the present study was performed by using Excel programs and statistically available software; Statistical Package for the Social Sciences (SPSS) Version 19 by using the Pearson correlation method. All values were expressed as mean ± standard deviation (SD). The differences were considered significant when the probability (P) was less than \( P \leq 0.05 \).

4- Results

Table [1] shows the baseline and demographic characteristics of the subjects of the study population. The Majority of the continuous hemodialysis patients and the apparently healthy control subjects were males (42 and 43) years age respectively of the total (80 and 85) years age respectively. The statistical analysis showed no significant differences between them in the present study with a mean age of (48.96 ± 5.18) years for the patients and 40.68 ± 9.58 years for the controls. The mean body mass index (BMI) was 20.94 ± 3.29 kg/m\(^2\) for the patients and 25.74 ± 1.43 kg/m\(^2\) for healthy control. On comparing to the anthropometric data, no significant difference (\(P>0.05\)) was observed between the cases and the controls with respect to their age and BMI (height, weight). The average duration of hemodialysis (HD) therapy for the CRF patients in the present study was 2.19 ± 0.76 years.

![Figure (1): The demographic data of the Study (Age, BMI) in continuous hemodialysis patients and in control](image)

Table (1): The demographic data of the Study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Continuous Hemodialysis patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (mean ± SD)</td>
<td>46.15 ± 16.72</td>
<td>43.59 ± 15.37</td>
</tr>
<tr>
<td>BMI (Kg/m(^2)) (mean ± SD)</td>
<td>21.41 ± 1.66</td>
<td>25.45 ± 1.83</td>
</tr>
<tr>
<td>Duration of HD (years) (mean ± SD)</td>
<td>2.43 ± 0.39</td>
<td></td>
</tr>
</tbody>
</table>

A significant changes in serum of the diagnostic parameters of kidney functions; blood urea (BU), serum creatinine (Cr) and creatinine clearance (CrCl) in the continuous hemodialysis patients in comparison to healthy control, as shown in table (2). There is a significant increase (\(P<0.05\)) in concentration of blood urea and serum creatinine, while the creatinine clearance was significantly decreased (\(P<0.05\)) in patients with continuous hemodialysis patients when compared to healthy control.

Table (2) : The renal function tests [Blood Urea (BU), Serum Creatinine (SCr) and Creatinine Clearance (CrCl)] in Patients and healthy control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Continuous hemodialysis patients (mean ± SD) n= 80</th>
<th>Median (mini-max)</th>
<th>Healthy control (mean ± SD) n= 85</th>
<th>Median (mini-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BU (mmol/L)</td>
<td>24.03 ± 1.04*</td>
<td>23.95 (22.00 - 27.00)</td>
<td>4.31 ± .89</td>
<td>4.00 (3.00 - 6.10 )</td>
</tr>
<tr>
<td>SCr (µmol/L)</td>
<td>211.03 ± 7.03*</td>
<td>212.00 (199.00 - 221.00)</td>
<td>87.02 ± 5.86</td>
<td>88.00 (78.00 - 100.00 )</td>
</tr>
<tr>
<td>CrCl (ml/min)</td>
<td>57.84 ± 2.64*</td>
<td>58.00 (50.00 - 62.00)</td>
<td>118.23 ± 10.44</td>
<td>122.00 (96.00 -133.00 )</td>
</tr>
</tbody>
</table>

*P < 0.05 Significant
A significant changes in the enzymes; serum paraoxonase PON-1, ̵-nucleotidase (5-NT), amylase and lipase activity in continuous hmodialysis patients in comparison to the healthy control subjects as shown in Table(3). There is a significant decreased (P<0.05) in PON-1 in patients with continuous hmodialysis when compared to healthy control. However, the serum 5-NT and amylase and lipase activities were significantly increased (P<0.05) in serum patients when compared with healthy control.

Table (3): The serum Paraoxonae (PON1), ̵-nucleotidase (5-NT), amylase and lipase and activity in continuous hemodialysis patients and in healthy control.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>CHD patients (mean ± SD) n=80</th>
<th>Median (min-max)</th>
<th>Controls (mean ± SD) n=85</th>
<th>Median (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1 (IU / L)</td>
<td>53.85 ± 3.52*</td>
<td>54.00 (44.00-58.00)</td>
<td>165.56 ± 7.43</td>
<td>168.00 (143.00-77.00)</td>
</tr>
<tr>
<td>5-NT (IU / L)</td>
<td>17.72 ± 0.43*</td>
<td>17.80 (15.00 - 18.00)</td>
<td>13.44 ± 0.27</td>
<td>13.50 (13.00 - 14.00)</td>
</tr>
<tr>
<td>Serum amylase (IU/L)</td>
<td>114.2 ± 5.09*</td>
<td>115.85 (100.00-120.00)</td>
<td>43.12 ± 2.16</td>
<td>43.00 (38.90 - 49.00)</td>
</tr>
<tr>
<td>Serum lipase (IU/L)</td>
<td>91.05 ± 1.62*</td>
<td>90.80 (87.50-94.10)</td>
<td>43.12 ± 2.16</td>
<td>43.00 (38.90-49.00)</td>
</tr>
</tbody>
</table>

*P < 0.05  Significant

A significant changes in the biochemical parameters; serum iron (Fe), total iron binding capacity (TIBC) and ferritin in the continuous hemodialysis patients in comparison to the healthy control as in Table (4). There is significant decreased (P<0.05) in Fe and TIBC concentrations in the serum of patients with continuous hemodialysis patients when compared to healthy control. While the serum ferritin was significantly increased (P<0.05) in these patients when compared to healthy control.

Table (4): Serum iron (Fe), total iron binding capacity (TIBC), ferritin in the continuous hemodialysis patients in comparison to healthy control.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>CHD patients (mean ± SD) n=80</th>
<th>Median (min-max)</th>
<th>Controls (mean ± SD) n=85</th>
<th>Median (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe (μmol/L)</td>
<td>10.89 ± 0.533*</td>
<td>10.80 (10.10- 11.90)</td>
<td>19.84 ± 1.83</td>
<td>19.30 (60.00 - 69.20)</td>
</tr>
<tr>
<td>TIBC(μmol/L)</td>
<td>36.87 ± 1.57*</td>
<td>37.00 (33.20 - 39.30)</td>
<td>65.46 ± 2.59</td>
<td>66.10 (60.00 -68.29)</td>
</tr>
<tr>
<td>Serum ferritin (ng/ml)</td>
<td>146.27 ± 2.96*</td>
<td>145.60(143.50-156.00)</td>
<td>116.76 ± 2.23</td>
<td>116.70 (100.80 - 122.50)</td>
</tr>
</tbody>
</table>

*P < 0.05  Significant
Table 5 explains the significant changes in the hematological parameters; hemoglobin (Hb), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), red blood cells count (RBCs) and white blood cell count (WBCs) in the continuous hemodialysis patients in comparison to the healthy control. There is a significant decreased (P ≤ 0.05) in (Hb), (PCV) and (RBCs) concentrations in patients with continuous hemodialysis when compared to healthy control. While (WBCs) and (ESR) were significantly increased (P ≤ 0.05) in these patients when compared to healthy control.

Table (5): hemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBCs), red blood cells count (RBCs) and erythrocyte sedimentation rate (ESR) in the continuous hemodialysis patients (CHD) and in control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CHD patients (mean ± SD)</th>
<th>Median (mini-max)</th>
<th>Controls (mean ± SD)</th>
<th>Median (mini-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (gm/dl)</td>
<td>8.80 ± 0.46*</td>
<td>8.80 (7.80 - 9.80)</td>
<td>13.14 ± 0.609</td>
<td>13.30 (12.00 - 14.20)</td>
</tr>
<tr>
<td>PCV %</td>
<td>28.32 ± 1.50*</td>
<td>28.30 (25.10 - 31.50)</td>
<td>42.29 ± 2.00</td>
<td>42.80 (38.50 - 45.70)</td>
</tr>
<tr>
<td>WBCs (cell/mm³)</td>
<td>6.56 ± 0.41*</td>
<td>6.50 (5.80 - 7.80)</td>
<td>5.84 ± 0.39</td>
<td>5.80 (4.70 - 6.60)</td>
</tr>
<tr>
<td>RBCs (cell/mm³)</td>
<td>3.30 ± 0.20*</td>
<td>3.30 (2.90 - 3.80)</td>
<td>4.66 ± 0.31</td>
<td>4.70 (4.00 - 5.20)</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>68.09 ± 2.06*</td>
<td>68.00 (65.00 - 71.00)</td>
<td>7.05 ± 1.37</td>
<td>7.00 (5.00 - 10.00)</td>
</tr>
</tbody>
</table>

*P < 0.05 Significant

Table (5): hemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBCs), red blood cells count (RBCs) and erythrocyte sedimentation rate (ESR) in the continuous hemodialysis patients and in control.

5- Discussion

The results showed a significant increased in the screening or diagnostic tests of renal function (BUN and SCR) (P > 0.05) as shown in the table [2]. The serum urea increasing in chronic hemodialysis is proportional to the progression of the disease, which is highly influenced by a catabolic state or an excessive protein ingestion, leading to a higher production of other waste substances of protein catabolism [38] while the increase in creatinine level in the serum of patients with CRF is attributed to the decrease in the number of functioning nephrons, which would reduce the GFR, which causes major decrease in renal excretion of water and solutes [39] these results are compatible with Khalidah S. Merzah [2015][40]. The Cr is cleared from the body by the kidneys, when the kidney functions abnormally, Cr concentration increases in the blood because little amount of it is released through the urine so that there was a significantly reduction in the CrCl [37]. PON-1 activity showed a significant decreased (P > 0.05) as shown in table [3] which is compatible with Ferenc Szatánk, e tal (2012) [41] and Emtedhar R. Sarhat1, e tal. (2017)[42].The activity of PON-1 is lower in renal insufficiency patients (chronic peritoneal dialysis; chronic hemodialysis; chronic renal failure) than in healthy control subjects[12]. The possible causes of reduced paraoxonase activity in uremic patients can include reduced HDL levels, altered HDL subfraction distribution, reduced PON1
concentration and different paraoxonase phenotype distributions. Another possible explanation could be that paraoxonase activity is inhibited in the uremic environment. However, the cause of the reduction in paraoxonase activity in CRF remains unclear [43]. Table (3) revealed also a significant increased in the concentration of ‘5-NT (P > 0.05). This result is in correspondence with other studies which show a highly increase in ‘5-NT activity in the serum of blood of continuous hemodialysis patient when compared to the healthy control. It has been suggested that increase ‘5-NT activity in these patients due to the existence of alterations in nucleotide hydrolysis in continuous hemodialysis patients. This altered nucleotide hydrolysis could participate in homeostasis abnormalities found in chronic hemodialysis [44,45]. Also there is a significant increased in the concentration of serum amylase and lipase in patients with continuous hemodialysis (P > 0.05). These pancreatic enzymes are mainly filtered by the kidney and are thereafter reabsorbed at varying rates [46]. Causing significant decreased in the clearance of these enzymes in patients on hemodialysis, levels of these enzymes are often increased in these patients[47]. The degree of the increasing is nearly proportional to the degree of renal impairment but the absolute values do not exceed three times the upper limit of normal. The serum lipase is increased due to renal impairment and by the dialysis procedure itself in patients with ESRD, possibly related to the use of heparin[48].

This result is in agreement with Fahrenkurg I, e tal. (1981)[49]. Serum Fe and TIBC levels were found to be significantly decreased (p<0.05) in serum of the patients compared with the control group as shown in Table (4). These results are compatible with Israa A .M. Jumaaah. (2013)[50]. The kidneys produce erythropoietin, calcitriol and renin, as a part of the endocrine system function. Erythropoietin partakes in the production of red blood cells and calcitriol plays an important role in the bone formation[51]. In regular hemodialysis patients: the kidneys are unable to secrete erythropoietin (EPO ) hormone, which is a necessary stimulus for normal bone marrow to produce red blood cells. Accumulation of uremic toxins, may play a role in depressing bone marrow function so that, anemia of chronic hemodialysis[52] is a result of a decreased production of the RBCs by the bone marrow [52] as shown in Table (5). EPO stimulates the action of bone marrow to make (RBCs), When the kidneys are damaged as in chronic hemodialysis, they do not make enough EPO. As a result, the bone marrow makes little (RBCs), causing anemia. Other common causes of anemia in patients with hemodialysis include blood loss from hemodialysis and low levels of the following nutrients found in food: iron, vitamin B12,folic acid which are necessary for red blood cells to make hemoglobin, the main oxygen-carrying protein in the (RBCs)[53]. The concentration of ferritin increased in patients with continuous hemodialysis when compared with healthy control groups because iron is not utilized because there is less erythropoiesis (formation of red blood cells & hemoglobin). Thus, to store the unused iron, ferritin protein that binds this iron is produced in increased amount[54]. These results are compatible with Goodnough, L.T., et al. (2000)[55].Elevation of the erythrocyte sedimentation rate (ESR) is mostly due to inflammation and infection as a result of the vascular disease and catabolic processes occurring in CRF as a cause or complements, which are most probably due to elevated circulating levels of inflammatory cytokines such as interleukin-6[56].

Conclusion

From this study we can conclude that the serum paraoxonase PON -1 activity is the most commonly affected among biochemical parameters in chronic hemodialysis patients. Anemia is the common complication of chronic renal diseases due to deficient renal production of EPO.

References

دراسة بعض المتغيرات الكيميائية والدموية في مرضى الفشل الديمدي المستمر في مدينة كركوك

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تم الدراسة الحالية على 80 مريض للفشل الديمدي المستمر (42 ذكر، 38 أنثى، بعمر (20-70) سنة للبالغين في وحدة الفشل الكلوي في مستشفى كركوك العام بكركوك، Iraq. ونُفذت الدراسة بواسطة بعض المتغيرات الكيميائية مثل الإيروبييدين، البروتينات، الكرياتينين، الحديد، سعة ارتباط الحديد، الفيراتين، الالايبير، الكرياتينين، عدد الكرياتينين، عدد الفيراتين، والكليوبي، وعدد الكرياتينين، عدد الفيراتين، والكليوبي، وعدد الكرياتينين، عدد الفيراتين، والكليوبي، وعدد الكرياتينين، عدد الفيراتين، والكليوبي، وعدد الكرياتينين، عدد الفيراتين، والكليوبي، وعدد الكرياتينين، عدد الفيراتين، والكليوبي.

تُقدِّم الدراسة النتائج الإيجابية لزيادة معدلات بعض المتغيرات الكيميائية والدموية في مرضى الفشل الديمدي المستمر في مدينة كركوك. وتُظهر النتائج أن الدراسة تختلف في بعض المتغيرات الكيميائية والدموية، مما يشير إلى أن الفشل الديمدي المستمر له تأثيرات طويلة الأمد على صحة المرضى. هذا ي̀يلدDataset* نتائجًا إيجابية يمكن استغلالها في متابعة حالات الفشل الديمدي المستمر وتحديد مستقبل المرضى وتقديم الرعاية المطلوبة.

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