Study of some biochemical changes in serum of patients with chronic renal failure

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Abstract

The present study is concerned with the investigation of the activity of the folling enzymes adenosine deaminase (ADA), `5-nucleatidase (`5-NT) and amylase. The concentration of selenium (Se), iron (Fe), sodium (Na), potassium (K), peroxynitrite and vitamin A have been measured in blood serum from patients with chronic renal failure (CRF) undergoing hemodialysis .

Thirty-five patients with CRF before and after having hemodialysis and 62 healthy controls were included in this study.

The results showed a significant decreased in serum ADA activity of CRF patients while `5-NT and amylase activities were significantly increase in these patients when compared with blood serum from normal individuals. The concentration of Se, Fe, Na and vitamin A were significantly decreased while peroxynitrite was significantly increased. Potassium was increased, but the increase was statistically insignificant. In a comparative study in pre-and post hemodialysis cases, serum `5-NT activity and Na concentration in post hemodialysis patients were significantly decreased but the ADA activity and Fe concentration were significantly increased than those in the pre hemodialysis patients. There was no significantly difference in the serum activity of amylase and in the concentration of Se, K, peroxynitrate and vitamin A. In conclusion, hemodialysis patients exhibit some biochemical changes before and after the dialysis when compared with control group.

الخلاصة

شـهدت الدراسـة الحاليـة قيـاس فعاليـة بعـض الأنزيمـات والتـي تـشمل الأدينوسـين دي أمينيـز و 5` نيكليوتايديز والأميليز كذلك قياس تراكيز بعض العناصر مثل السلينيوم والحديد والصوديوم والبوتاسيوم وبعض

مضادات الأكسدة مثل البيروكسي نيتريت وفيتامين A في مصل المرضى المصابين بالعجز الكلوي المزمن والمعالجين بالديلزة الدموية.

أجريت الدراسة على 35 مريض مصابين بالعجز الكلوي تراوحت أعمارهم بين 30−70 سنة حيث تم سحب الدم من المرضى قبل إجراء عملية الديلزة الدموية وبعدها ومقارنة النتائج مع 62 عينة دم من أشخاص أصحاء كمجموعة سيطرة.

تشير النتائج إلى وجود انخفاض معنوي في فعالية أنزيم ADA بينما تظهر ارتفاعاً معنوياً في فعالية 5-NT` والأميليز في مصل دم المرضى المصابين بالعجز الكلوي مقارنة مع مجموعة السيطرة, كما لوحظ أن تراكيز كل من Na, Fe, Se وفيتامين A قد أظهرت انخفاضاً معنوياً بينما البيروكسي نتريت فقد أظهر زيادة معنوية. أما فيما يتعلق بالبوتاسيوم فقد وجد أنه قد ازداد زيادة غير معنوية.

عند دراسة المقارنة بين المرضى قبل وبعد إجراء الديلزة الدموية فإن النتائج تشير إلى أن فعالية أنزيم-5` NT وتركيز Na قد انخفضت إنخفاضاً معنوياً عند المرضى بعد إجراء عملية الديلزة الدموية بينما أظهرت فعالية ADA وتركيز Fe زيادة معنوية مقارنة مع المرضى قبل إجراء عملية الديلزة الدموية. كما تبين النتائج عدم وجود تغيراً معنوياً في فعالية أنزيم الأميليز وفي تراكيز كل من K, Se والبيروكسي نيتريت وفيتامين A . نستنتج من ذلك أن المرضى المصابين بالعجز الكلوي المزمن لديهم بعض التغيرات الكيموحيوية قبل وبعد إجراء عملية الديلزة عند مقارنتهم مع مجموعة السيطرة.

Introduction

Chronic renal failure (CRF) is long-standing, progressive deterioration of renal function. Symptoms develop slowly, diagnosis is based on laboratory testing of renal function, sometimes followed by renal biopsy. Treatment is primary directed at the underlying condition but includes fluid and electrolyte management and after dialysis and/or transplantation [(1)].

The CRF can be roughly categorized as mild, moderate, severe, or end-stage renal disease, hemodialysis or peritoneal dialysis is initiated as the glomerular filtration rate (GFR) falls. Some patients with CRF eventually receive kidney transplantation before (a few cases) or after (majority of recipients) initiation of hemodialysis or peritoneal dialysis. Initially, as renal tissue loses function, there are few abnormalities because the remaining tissue increases its performance (renal function adaptation), a loss of 75% of renal tissue produce a fall in GFR to only 50% of normal [(2)].

The CRF refers to decline in the GFR caused by a variety of disease, such as diabetes, glomerulone phritis, and polycystic kidney disease [(3)].

The progression of renal disease from one stage to the next, the need for

emergent or maintenance dialysis, prevention and management of fluid, electrolyte, and acid base imbalances before and after surgery, and the high cardiac risk are issues, that must be addressed before patient with CRF proceed for elective surgery The key to dialysis is the provision of a semipermeable membrane through which ions and small molecules, present in plasma at high concentrations of a rinsing fluid [4].

It is important to consider oxidative stress as a potentially important source of patient morbidity and mortality, although this knowledge is not yet immediately applicable in the clinical arena. Further well – designed, randomized controlled clinical trials with anti – oxidants (e.g. Vitamins) are required to establish evidence – based recommendation for clinical practice[5].

Reactive oxygen specias, especially ($O^{\circ}2^{-}$) , is elevated in patients with CRF, and their antioxidant capacity is decreased. Salt- sensitive have progressive decreases in renal function. Renal $O^{\circ}2^{-}$ cause progressive increases in renal damage and decrease renal hemodynamics. Several antioxidant regimens such as vitamins decrease renal tissue $O^{\circ}2^{-}$ production, prevent renal damage, increase renal hemodynamics [6].

The aim of this study was to evaluate the effect of hemodialysis on some biochemical changes before and after the dialysis and compared with control group.

Materials and Methods

Subjects and Methods

This research was conducted on a random sample of patients with CRF who attended the artificial kidney unit in Ibn-Sina Hospital in Nineveh Governorate, and they account 35 patients (15 males and 20 females) ranging in their age between 30-70 years. These samples were diagnosed clinically and radiologically as having chronic renal failure undergoing hemodialysis. Blood serum from these patients was freshly withdrawn by vene-puncture of each patient before and after hemodialysis. Serum then was separated by centrifugation at 3000xg for 10 minutes, and then divided in aliquots each subject's serum was frozen at (-20 ºC) before analysis [7] Blood samples from 62 control subjects were obtained for comparison

The ADA activity was determined according to Guisti method [8]. The `5-NT activity was measured

by following Wood and Williams method [9]. Amylase activity was determined according to Wootten method [10]. Selenium concentration was estimated by using the colorimetric method [11]. Iron in serum was assayed by using atomic absorption spectroscopy technique [7]. Sodium and potassium in serum were determined using flam emission spectrometry [7]. Peroxynitrate level was estimated using the modified method of Menden et al., [12]. Vitamin A was determined by colorimetric method [10].

The results were statistically analyzed using the number, percentage and Z-test between two proportions. By comparing biochemical factors between control group and patients group, pooled t-test or unpaired Z-test were used according to the number of samples. All the data were expressed as mean ± standard error of the mean. The results were considered significantly at $P<0.05$.

Results and Discussions

The results In Table (1) showed that there is a significant decrease ($p<0.001$) in ADA activity in serum of patients with CRF in a pre and post hemodialysis patients, in comparison with control. The percent decrement for pre and post hemodialysis patients was about (51% and 39%) respectively compared with control.

Also the results in Table (2) showed that there is a significant increased ($p<0.05$) in ADA activity in post hemodialysis patients than those in the prehemodialysis state.

The results of the present study were agree with those obtained by [13] [14]. The decrement of ADA activity from patients with CRF exhibited an increased rate of ATP formation from adenine as a substrate. Thus, we concluded that this process was in part responsible for the increase of adenine nucleotide concentration in uremic erythrocytes. These cannot be excluded however , that a decreased rate of adenylate degradation is an additional mechanism responsible for the elevated ATP concentration. Also the deficiency of ADA activity leads to severe immunodeficiency disease in which T-lymphocytes and B-lymphocytes do not develop properly, that is mean the ADA is a non-specific marker of the activation of the T and B cells, which has an important role in the etiology of several disease [15].

Other studies., noted that hemodialysis patients have high concentration of adenosine in serum due to a decrease in ADA activity. The

uptake of adenosine by blood ADA activity is normal in hemodialysis patients, suggesting that no other metabolic pathways are altered. Also they showed that adenosine concentrations and low ADA activity are involved in the dialysis induced immunodeficiency in hemodialysis patients. Indeed adenosine and its metabolites deteriorate lymphocyte function and proliferation in a dose-dependent manner and low ADA activity not only increases the intracellular adenosine concentration but also inactivates T cells [16].

In this study, pre and post hemodialysis serum activity of `5-NT enzyme was measured in patients with CRF. The activity was found to be higher in pre and post hemodialysis patients when compared to the controls, as shown in Table (1). The percentage of increment was (35% and 19%) in pre and post hemodialysis respectively. Highly significant difference ($P<0.001$) was found however between pre and post hemodialysis values for enzyme in the patient group, as shown in Table (2).

These results are in corresponding with other studies which show a highly increase in `5-NT activity in serum of CRF patient when compared to the control. It has been suggested that increase `5-NT activity in serum of CRF patients accrue to the existence of altrations in nucleotide hydrolysis in CRF patients. Possibly, this altered nucleotide hydrolysis could contribute to hemostasis abnormalities found in CRF [13][17].

The results in Table (1) showed a significant increased ($P<0.001$) in amylase activity in serum of pre and post hemodialysis patients in comparison in control group. The percent increment was about (43% and 35%) in the two groups of patients respectively in comparison with controls group.

These were several results, which were conformable to our results of amylase activity in CRF patients [18] [19], which they observed that serum amylase activity was three times greater than the upper limit on normal. Also they noted that patients exhibit a marked elevation of serum amylase level in the absence of clinical pancreatitis. The observed hyperamylasemia is not associated with increased P3 isoamylase level unless pancreatitis is present [18]. Also, the elevation in serum amylase among patients with renal failure or end-stage renal disease is most likely due to impaired renal clearance. In one study, the serum amylase began to rise only

when the creatinine clearance dropped below 50 mL/min [19].

On the other hand the results in Table (2) show that there are no significant differences between pre and post hemodialysis patients of amylase activity. This result was in accordant with other studies which they reported that the dialysis procedure alone does not appear to alter serum amylase. In one study [18], for example, no change was observed in serum amylase in samples obtained pre and post dialysis. However, these observations are confounded by the failure to the failure to correlated levels of amylase with variations in dialysis membrane clearance [19].

The trace mineral selenium was determined in this study, which is an essential nutrient of fundamental importance to human biology . The result of Se concentration was shown in Table (1) which indicate that there is a significant decreased ($p<0.001$) in pre and post hemodialysis patients when compared with control group. The percentage of decrement was about (33%and 26%) respectively.

Similar published results showed thet low Se concentration in serum of patients with CRF [20].

There are a number of hypotheses that have been postulated to account for the experimental data that Se reduced disease. They are: Selenium's enzyme, Seleniym's enhancement of immunity, Selenium's effect on the metabolism, and selenium's interactions that affect protein synthesis and cycle of cell division [21].

Other studies showed low selenium concentration and attributed their finding to the acting of selenium as antioxidant by binding with vitamin E and as a constituent of glutathion peroxidase to scavenge free radical to detoxify tissue per oxidation [22].

Recently, Margaret. Has been proposed that exposure to an increased oxidative burden related to inflammation and diseases such as immunodeficiency and cardiovascular disease play an important role in the pathogenesis of the associated diseases, particularly in hemodialysis patients [23].

Indeed, in addition to an excess generation of reactive oxygen species (ROS), uraemic patients have a decreased antioxidant capacity, which causes oxidative damage to cells [24].

The results in Table (2) show that there are no significant differences between pre and post hemodialysis patients in Se concentration.

Table (1): The enzymes activity and concentration of the parameters in blood serum of hemodialysis patients and control subjects

Parameters	Mean ± SE		
	Control n=62	Pre hemodialysis patients n = 35	Post hemodialysis patients n = 35
ADA U/L	1.69 ± 0.03	0.83 ± 0.08 ***	1.03 ± 0.07 ***
`5 – NT U/L	13.12 ± 0.40	17.65 ± 0.23 **	15.66 ± 0.35 **
Amylase U/dl	6.11 ± 0.15	8.74 ± 0.32 ***	8.27 ± 0.31 ***
Se µmol/L	0.82 ± 0.04	0.55 ± 0.02 ***	0.61 ± 0.03 ***
Fe µmol/L	18.85 ± 0.54	10.24 ± 0.49 ***	13.67 ± 0.51 ***
Na mmol/L	138.26 ± 1.01	136.43 ± 1.11	122.86 ± 4.18 ***
K mmol/L	3.82 ± 0.1	4.35 ± 0.19	4.18 ± 0.27
Peroxynitrit µmol/L	61.18 ± 3.0	85.67 ± 6.32 **	83.39 ± 4.76 **
Vitamin A µmol/L	1.82 ± 0.05	0.72 ± 0.08 ***	0.78 ± 0.03 ***

***** Significant difference between pre hemodialysis at (p< 0.001)**
**** Significant difference between pre hemodialysis at (p< 0.01)**
*** Significant difference between pre hemodialysis at (p< 0.05)**

Compared with control group, Iron concentration was found to be significantly decreased ($p<0.001$) in serum of pre and post hemodialysis patients as shown in Table (1). The decrement percent was about (46% and 27%) respectively when compared with control group.

The results were similar to that found by [25] which they indicated that renal dysfunction may give rise to a variety of hematologic disturbances, including anemia, leukocyte dysfunction, and coagulopathy.

The anemia of renal failure has been attributed to a relative deficiency of erythropoietin, but absolute deficiencies of iron or folate may also play a role. Other contributing factors include heavy- metal toxicity, blood loss, and a reduction in red cell survival induced by toxic radicals. The treatment of the anemia of renal disease has advanced with the development of recombinant human erythropoietin [26].

Table (2): The enzymes activity and concentration of the parameters in blood serum of pre and post hemodialysis patients.

Parameters	Mean ± SE	
	Pre hemodialysis patients n = 35	Post hemodialysis patients n = 35
ADA U/L	0.83 ± 0.08	1.03 ± 0.07 *
`5 – NT U/L	17.65 ± 0.23	15.66 ± 0.35 ***
Amylase U/dl	8.74 ± 0.32	8.27 ± 0.31
Se µmol/L	0.55 ± 0.02	0.61 ± 0.03
Fe µmol/L	10.24 ± 0.49	13.67 ± 0.51***
Na mmol/L	136.43 ± 1.11	122.86 ± 4.18 **
K mmol/L	4.35 ± 0.19	4.18 ± 0.27
Peroxynitrite µmol/L	85.67 ± 6.32	83.39 ± 4.76
Vitamin A µmol/L	0.72 ± 0.08	0.78 ± 0.03

***** Significant difference between pre hemodialysis at (p< 0.001)**
**** Significant difference between pre hemodialysis at (p< 0.01)**
*** Significant difference between pre hemodialysis at (p< 0.05)**

Henry explained the physiologic chemistry of Iron which it has the potential to cause deleterious effects by formation of toxic oxygen radicals that can attack all biological molecules [27]. In addition, iron has multiple effects on cell-mediated immunity by modulating the prolifer-ation and differentiation of lymphocyte subsets and by affecting the immune potential of macrophages that is, iron mediated inhibition of interferon - directed immune response pathways in macrophages.

On the other hand, iron can participate in a number of reactions to produce free radical species, which can damage cellular constituents. Therefore, tight regulation of iron homeostasis is an absolute requirement for maintaining essential cellular functions [27].

The concentration of Fe remains low after post hemodialysis in CRF patients When compared with control groups but higher than the pre hemodialysis stage. This results may be due to the therapeutic drugs which can activate the bone marrow's functional ability to produced the red blood cells. This results as shown in Table (2) were conformable to other results which they noted that there is a significant increased in post dialysis when compared with pre dialysis patients in iron concentration [28].

Sodium and potassium concentrations were determined in serum of patients with CRF, and the results show that there was a significant decreased ($p<0.001$) in post hemodialysis patients of sodium concentration when compared with

control group. The percentage of decrement was about (11%), while there is no significant changes in serum of pre hemodialysis patients when compared with control group as shown in Table (1).

There are several common causes of hyponatremia that may be grouped into three main categories renal loss, extrarenal loss , or cellular shift. The use of thiazide diureties induces sodium loss without interfere-ing with antidiuretic hormone (ADH). Mediated water retention. Aldost-erone deficiency increases renal loss of sodium and water, with sodium loss in excess of water loss. In diabetes meltitus, sodium loss occurs with ketonuria. Furthermore, salt-losing nephropathy may infrequently develop in renal tubular and interstitial diserses, such as medullary cystic and polycystic kidney diseases, usually as renal insufficiency becomes severe. Hypovolemic hyponatremia accompanied by a urinary sodium is caused by extra renal loss of hypotonic fluid as with prolonged vomiting, diarrhea , sweating or trauma [29].

The kidneys play a critical role in regulating electrolytes. They control the levels of sodium and potassium. Therefore, a disturbance in blood levels of these electrolytes may be related to kidney function [30].

Also, the result in Table (2) showed that there is a significant decrease ($p<0.01$) of sodium concentration in post hemodialysis patients when compared with pre hemodialysis. Most CRF patients retain the ability to reabsorb sodium ions, but the renal tubules may lose their ability to reabsorb water and so concentrate urine. Polyuxia, although present, may not be excessive because the GER is so low. Because of their impaired ability to regulate water balance, patients in renal failure may become fluid overloaded or fluid depleted very easily [31].

The results in Table (1) showed that there are no significant differences in pre and post hemodialysis patients of potassium concentration when compared with control group. Also the no significant changer in serum of patients with CRF as shown in Table (2).

Under normal conditions, the serum potassium level is (3.5 to 5.0 μmol/L). The consequences of potassium excess or potassium deficit can be catastrophic. Fortunately, the body has tightly regulated homeostatic mechanisms for maintaining normal total potassium content and a normal ratio of intracellular to extracellular potassium [30].

This research included the assessment of generation of oxygen reactive species via determination the level of peroxynitrite radical (ONOO) in patients with CRF. The results showed that there is significant increased ($p<0.01$) in patients with CRF when compared with control group, The percent increment was about (40% and 36%) respectively, as shown in Table (1).

The reaction between nitric oxide (NO°) and led to formation of peroxynitrite, which can caused lipid peroxidation, base modifiction, stand breaks, cysteine oxidization and dityrosyl- bridges formation. Decomposition of peroxynitrite is suggested to proceed through peroxynitrous acid. NO° can also can decompose ONOOH. By these mechanisms, NO° serves to abate the oxidation chemistry of RNOS [32].

The role of NO° is ubiquitous. NO° was reported to inhibit cell proliferation, to induce differentiation, In addition NO° is a reactive compound and can react with other free radicals such as superoxide ($O^{\circ}2^{-}$) and may cause the production of the

more destructive compound (preoxy-nitrite). Accordingly, it may be suggested that oxidant stress and NO may have multiple effects on the intiation. In addition, NO˚ can be a very effective antioxidant to the reactive oxygen species (ROS). The antioxidant mechanisms is through the versatile chemistry of NO˚ with ligand- metal and radical- radical. Because of the extremely complexity in nitric oxide function and mechanism [32].

Also, the results in Table (2) showed that there was no significant difference in peroxynitrite concentration before and after hemodialysis of patients with CRF.

In the follow- up study and after hemodialysis patients, vitamin A concentration in patients with CRF was significantly decreased ($p<0.001$) than those in the control group. The percentage of decrement was about (60% and 57%) in pre and post hemodialysis patients respectively, as shown in Table (1).

Many studies suggest that high blood levels of vitamin A can help prevent certain diseases such as CRF [33]. Also *Orman etal*. showed a tendency to decrease in serum of vitamin A in patients of CRF before and after treated by hemodialysis [5]. This may be due to that retinol and it analogues acts as inhibitors of superoxide radical production in these disease [34].

The increased risk of CRF in vitamin A deficiency is thought to be the result of a depletion in β- carotion , this compound is a very effective antioxidant and is suspected to reduce the risk of disease known to be initiated by production of free radicals. Of particular interest is the potential benefit of increased β- carotene intake to reduce the risk of CRF [5].

The largest study of the data on dietary risk factors reinforces earlier finding that consumption of fruit and vegetables which contain vitamin A reduces risk of developing the disease. Also, they concluded that in maintenance hemodialysis patients, vitamins administration resulted in a significant increase in the post dialysis level of the oxidized from of vitamins, which suggested an increase in antioxidant effect [35].

On the other hand the result in Table (2) indicated that there is no significant changes in vitamin A concentrations in pre and post hemodialysis patients.

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