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Evaluation of Serum Paraoxonase and Lipid Profile in Patients with Chronic Renal Failure Pre and post Hemodialysis

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Abstract

Chronic Renal Failure (CRF) is the progressive and irreversible loss of normal functioning of kidneys. this is associated with increased levels of some biochemical parameters and decreased others. This study aims to evaluate the value of serum paraoxonase-1 (PON1) activity as biochemical marker for patients with chronic renal failure (CRF) before and after hemodialysis (HD).

The samples of this study consists of 100 patients (55 males, 45 females) and 100 apparently healthy individuals (57 males, 43 females) from 20 - 70 years old of age. All patients included in this study were those who were receiving hemodialysis therapy in the dialysis unit in Kirkuk general Hospital from the period between February 2013 and May 2014. Serum PON1, BU, Cr, TC, TG, HDL, LDL levels were measured by spectrophotometric methods.Paraoxonase1 activity level was significantly lower in cases of CRF patients than in normal healthy control in pre hemodialysis (HD). It's level was significantly increased in post HD ($P > 0.05$). The blood urea (BU), serum creatinine (Cr), total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were significantly higher than normal healthy control in pre HD ($P < 0.05$), In post HD their levels were significantly decreased as compared to the pre HD ($P < 0.05$) but still lower than normal control, the serum high density lipoprotein (HDL) of CRF patients was significantly lower than normal controls ($P < 0.05$) in pre HD and was significantly increased in post HD as compared to pre HD but remained lower than normal healthy control. Conclusion: PON1 activity was significantly decreased in CRF patients before HD and significantly increased after HD but remained lower than controls. PON1 was significantly and negatively correlated with the age, BMI, duration of disease, BU, Cr, TC, TG, LDL, and VLDL. This study demonstrates that there is an increased risk of cardiovascular complications in CRF patients undergoing hemodialysis by increasing TC, TG, LDL, VLDL and decreasing HDL.

Introduction

Paraoxonase (PON1, EC 3.1.8.1) is a member of the paraoxonase family (PON1, PON2, PON3). PON1 is synthesized and secreted by the liver, and in circulation it is associated with high density lipoprotein (HDL) [1] .Several Studies have shown that PON1 levels in humans have a distribution characteristic of two alleles, one with low activity and the other with high activity.PON1 also has arylesterase activity, which does not exhibit it activity polymorphism and can therefore serve as an estimate of enzyme protein. Although the ability of paraoxon to irreversibly inhibit lipoprotein lipase (LPL) has been exploit experimentally for many years, the role of plasma paraoxonase in lipoprotein metabolism is unknown[2].PON1 is involved in the detoxification of organophosphate in some organophosphates (OPs) and its polymorphisms influence enzyme activity and quantity[3]it is serum protein, the activity of which is related to susceptibility to cardiovascular disease and intoxication by organophosphorus (OP) compounds. It may also be involved in innate immunity, and it is a possible lead molecule in the development of a catalytic bioscavenger of OP pesticides and nerve agents. Human PON1 expressed in E. coli is mostly found in the insoluble fraction, which motivated the engineering of soluble variants, such as G2E6, with more than 50 mutations from huPON1. All three sets of mutations increase the solubility of huPON1; the HDL - binding mutant has the largest effect on solubility, but it also decreases the activity and stability the most. Based on the G2E6 polar mutations, an engineered variant of PON1with high activity against cyclosarin (GF) and found that it was still very active against GF with much greater similarity to the human sequence[4]. Therefore, PON1 is the main means of protection of the nervous system against the neurotoxicity of organophosphates entering the circulation[5]. Human PON1 is not stable, and tends to aggregate in the absence of detergents. In addition, it cannot be expressed in bacteria or yeast for protein over-expression, mutagenesis and protein engineering. Therefore, this protein was submitted to directed evolution in order to over - express in E.coli and to increase its solubility. Mortality rates are higher among patients with chronic renal failure with a good proportion of this excess burden being attributable to cardiovascular disease. This excess risk is not entirely explained by elevated traditional risk factors. Among the non-traditional risk factors, serum paraoxonase (Arylesterase) activity is an important one[6]. Decreased serum arylesterase activity, catalyzed by the high-density lipoprotein – associated (PON)-1, is associated with increased oxidant stress and atherosclerosis risk [7]. Reduced serum PON1 activity has been clearly established in the past decade and could contribute to accelerated development of atherosclerosis in ESRD and in HD. PON1 lactonase activity is lower in ESRD patients.

Materials and Methods

A total of 100 patients (55 males, 45 females) and 100 healthy individuals (57 males, 43 females) from 20 - 70 years of age were studied. All patients were receiving hemodialysis therapy in the dialysis unit in Kirkuk public hospital from the period between February 2013 and May 2014.

After 12hrs fasting, pre dialysis venous blood samples were collected from all patients, by well trained and experienced nurses. Venous blood (6 ml) was drawn into plain tubes. The non - heparinized blood in the plain tubes were left (10 - 15 minutes) to clot and then centrifuged at 3000 rpm for 5 minutes then the separated serum put in the plastic tubes and stored at (-20C^{o}) until assayed. The process of sample collection was repeated post hemodialysis.

Paraoxonase activity assay was performed using paraoxon (O,O-diethyl-O-p-nitrophenyl phosphate; Sigma-Aldrich ,Germany) as a substrate according to the method described by Mackness *et al*[8].The rate of paraoxon hydrolysis were determined by measuring the released p-nitrophenol at 405 nm. Serum BU, Cr, TC, TG, HDL, LDL levels were measured by spectrophotometric kit, VLDL was measured by the equation: VLDL (mmol/L) =TG / 2.2

Results

The baseline and demographic characteristics of the subjects of the study population are shown in Table(1). The Majority of the chronic renal failure (CRF) patients and the control subjects were male (55 %and 57%) respectively of the total (100) equal number of the CRF patients and healthy subjects but statistical analysis showed no significant difference 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. 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.

Table (1) Demographic characteristics of study population

	Healthy controls	CRF patients
Age (years) (mean ± SD)	40.68 ± 5.18*	48.96 ± 9.58* 5.18*
Sex (males %) (females %) Total	57 43 100	55 45 100
BMI (Kg/m²) (mean ± SD)	25.74 ± 1.43*	20.94 ± 3.29*
Duration of hemodialysis (HD) (years) (mean ± SD) = 2.19 ± 0.76		

* $P > 0.05$ Not significant

Table(2) and (3) represent a significantly decreased in serum PON1 and increased in BU and serum Cr levels in patients with CRF in pre HD when compared with the control ($P < 0.05$). In post HD, PON1 level was significantly increased as compared with the pre HD but still lower than normal control, BU and serum creatinine levels were significantly decreased but remained at high levels when compared with control groups ($P < 0.05$).

Table (2) Levels of serum paraoxonase1 (PON1), blood urea (BU) and serum creatinine (Cr) in CRF patients pre and post (HD) and in healthy controls.

Biochemical Parameters	Healthy controls (Mean ± SD)	CRF patients Pre - hemodialysis (Mean ± SD)	CRF patients Post - hemodialysis (Mean ± SD)
Paraoxonase1 (IU / L)	149.41± 6.19	50.02 ± 6.63**	90.62 ± 9.14**
Bood urea mmol / L	4.53 ± 0.816	29.40 ± 6.40**	22.96 ± 5.70**
Serum creatinine mmol / L	0.83 ± 14.32	0.89 ± 15.317**	0.68 ± 16.30**

** $P < 0.05$ Significant

Table (3) Levels of serum paraoxonase1 (PON1), blood urea (BU) and serum creatinine (Cr) in CRF patients pre and post (HD).

Biochemical parameters	CRF patients Pre - HD (Mean ± SD)	CRF patients Post - HD (Mean ± SD)
Paraoxonase1(IU / L)	149.41± 6.19	50.02 ± 6.63**
Blood urea (mmol / L)	29.40 ± 6.40**	22.96 ± 5.70**
Serum creatinine (mmol / L)	0.89 ± 15.317**	0.68 ± 16.30**

** P < 0.05 Significant

Table (4) and (5) represent that there was a significantly increased in the serum TC, TG, LDL and VLDL levels in pre HD as compared to normal healthy control (P<0.05). Their levels were significantly decreased in post HD when compared with pre HD but still higher than normal level (P<0.05). HDL level was significantly decreased in pre HD when compared with normal healthy control (P<0.05). In post HD it's level was significantly increased as compared to pre HD but remained lower than normal healthy control (P < 0.05).

Table (4) Concentration of lipid profile in chronic renal failure (CRF) patients pre and post HD and in healthy controls

Parameters	Healthy Controls (Mean ± SD)	CRF patients Pre - HD (Mean ± SD)	CRF patients post - HD (Mean ± SD)
Total cholesterol (mmol/L)	4.35 ± 0.63**	7.16 ± 0.36**	6.50 ± 0.37**
Triglyceride (mmol/L)	1.15 ± 0.30**	3.41 ± 0.62**	2.77 ± 0.56**
HDL (mmol/L)	1.09 ± 0.14**	0 .62 ± 0.12**	0 .75 ± 0.12**
LDL(mmol /L)	3.54 ± 0.26**	5.03 ± 0.39**	4.25 ± 0.15**
VLDL(mmol /L)	0.52 ± 0.13**	1.55 ± 0.28**	1.26 ± 0.25**

** P < 0.05 Significant

Table (5) Concentration of lipid profile in chronic renal failure (CRF) patients pre and post HD.

Parameters	CRF patients Pre-HD (Mean ±SD)	CRF patients post-HD (Mean ±SD)
Total cholesterol mmol/L	7.16 ± 0.36**	6.50 ± 0.37**
Triglyceride mmol/L	3.41 ± 0.62**	2.77 ± 0.56**
HDL(mmol/L)	0 .62 ± 0.12**	0 .75 ± 0.12**
LDL (mmol/L)	5.03 ± 0.39**	4.25 ± 0.15**
VLDL (mmol /L)	1.55 ± 0.28**	1.26 ± 0.25**

** P < 0.05 Significant

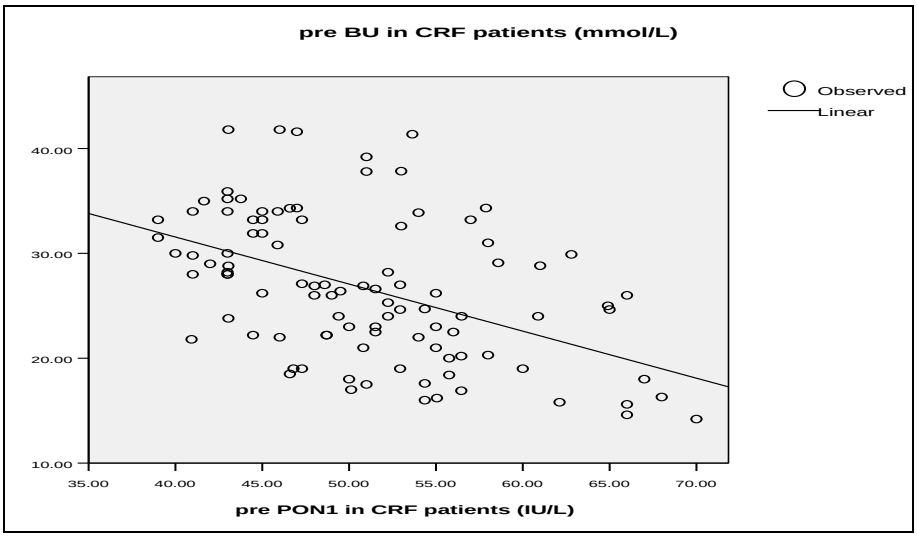

Figure (1) Correlation Coefficient Between Paraoxonase (PON1) and the BU of the CRF Patients Pre HD (R = - 0.477, P = 0.000)

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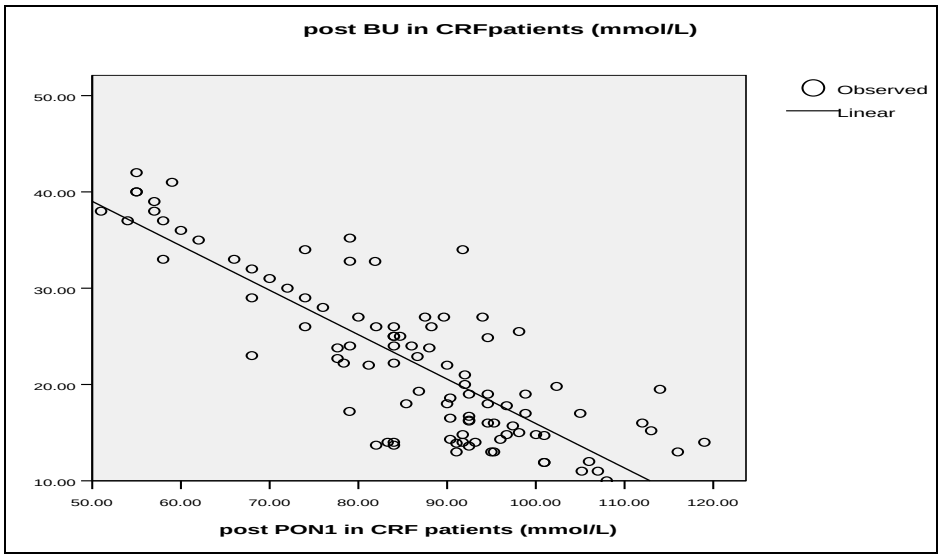

Figure (2) Correlation Coefficient Between Paraoxonase (PON1) and the BU of the CRF Patients Post HD (R = - 0.831, P = 0.000)

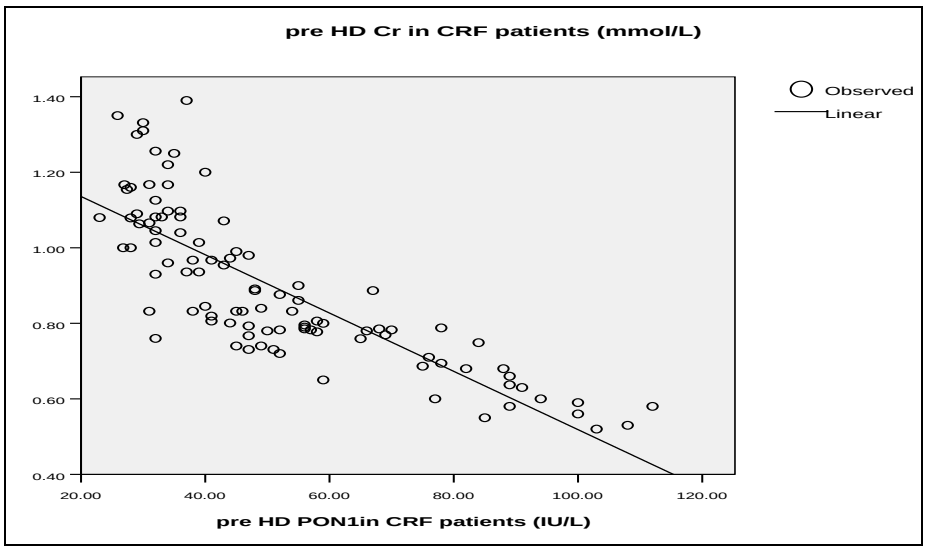

Figure (3) Correlation Coefficient Between Paraoxonase (PON1) and the Serum Cr of the CRF Patients Pre HD (R = - 0.843, P = 0.000)

Discussions

Paraoxonase is an enzyme associated with high density lipoprotein (HDL). It has been shown to prevent atherosclerosis by inhibiting oxidation of low density lipoprotein (LDL). Patients with chronic renal failure are at increased risk of developing atherosclerosis. Cardiovascular disease is the main cause of mortality and morbidity in patients with chronic renal failure(CRF) and in patients undergoing hemodialysis. Reduced activity of serum paraoxonase is considered to play a role in the development of atherosclerosis and other cardiovascular complications in these patients[9]. In this study and according to the table (4-2), the mean activity of paraoxonase-1(PON1) in controls and in patients with CRF during pre- and post- hemodialysis (HD) sessions were in the range of (149.41 ± 6.19) IU / L, (50.02 ± 6.63) IU / L and (90.62 ± 9.14) IU / L respectively. Increase in the activities of PON-1 have been evident during post - hemodialysis as compared to pre - hemodialysis samples of patients with CRF. The mean activity of PON-1 were decreased in pre - hemodialysis samples of patients with CRF as compared to healthy controls. PON-1 activity was increased in CRF patients during post - hemodialysis as compared to pre-hemodialysis sessions but it concentration still lower than the normal healthy controls level. In both, the results were statistically highly significant ($P < 0.05$). This is in accordance with the study of Kasat S. [10], Nagane N. et al[11]. and Gugliuccil A. [12].The decrease in PON-1 activity observed in this study could be the result of lower HDL-C concentrations in CRF given that HDL is the main serum carrier of PON-1. PON-1 inhibit the oxidative modifications of LDL during copper oxidation in vitro, possibly by destroying active phospholipids in minimally oxidized LDL. Serum PON-1 activity is inversely related to the risk of

developing atherosclerotic lesion which contains cholesterol loaded macrophage foam cells. Hemodialysis seems to be effective also in raising serum PON-1 activity of the patients. PON-1 present in serum is located on HDL, being tightly bound to a HDL subfraction containing apo A-I and clusterin. In CRF, the concentration of middle size and low molecular weight plasma Advanced Glycation End Products (AGE) are highly elevated. These AGE residues are formed on long and short lived proteins. Due to low molecular weight, AGE free adducts are easily excreted through the urine. Hence, it acts a good renal clearance tool which distinctly declines in CRF adduct. Retention of AGE free adducts could play a role in decreasing PON-1 activity.

Conclusion

1- PON1 activity was significantly decreased in CRF patients before HD and significantly increased after HD but remained lower than normal healthy control.

2- BU, Cr, were significantly increased in CRF patients due to the impairment of kidney function and reduced glomerular filtration rate (GFR).

3- This study demonstrates that there is an increased risk of cardiovascular
complications in CRF patients undergoing hemodialysis by increasing TC, TG, LDL, VLDL and decreasing HDL.

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دراسة فعالية أنزيم الباروكسينيز ومستويات الدهون لدى مرضى الفشل الكلوي المزمن

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الملخص

ويرتبط مع زيادة مستويات بعض القياسات الكيموحيوية وانخفاض البعض الآخر. غسيل الكلى هي إحدى البدائل العلاجية ، حيث يتم خلالها إزالة النواتج مثل اليوريا والكرياتينين والماء الزائد من الجسم.تهدف هذه الدراسة إلى تقييم فعالية أنزيم الباروكسينيز في مصل المرضى المصابين بالعجز الكلوي المزمن كعلامة بيوكيميائية لهؤلاء المرضى وكذلك قياس تراكيز اليوريا والكرياتينيين في المرضى المصابين بالعجز الكلوي قبل وبعد الديلزة الدموية ودراسة العلاقات المتبادلة بين كل من الباروكسينيز والقياسات البيوكيميائية المستخدمة في هذه الدراسة. عينات هذه الدراسة تتكون من 100 مريض (55 ذكور، 45 إناث) و100 أفراد ظاهريا أصحاء (57 ذكور، 43 إناث) يتراوح أعمارهم ما بين 20–70 سنة من العمر. جميع المرضى في هذه الدراسة أولئك الذين كانوا يتلقون علاج غسيل الكلى في وحدة الكلية الصناعية في مستشفى كركوك العام في الفترة ما بين شهر شباط عام 2013 وشهر آيار عام 2014. مصل الباروكسينيز واليوريا والكرياتينين وحامض والكولسترول والدهون الثلاثية والبروتين الدهني العالي الكثافة والبروتين الدهني الواطئ الكثافة تم قياس مستوياتهم بطرق طيفية. لقد اثبت الدراسة الحالية انخفاض معنوي في فعالية إنزيم الباروكسينيز في مصل المرضى المصابين بالعجز الكلوي المزمن قبل الغسل مقارنة بالمستوى الطبيعي له وتزداد فعاليته بعد الغسل ولكنه يبقى اقل من الحد الطبيعي. اليوريا والكرياتينين والبروتين الدهني الواطئ الكثافة والبروتين الدهني الواطئ الكثافة جدا والكولسترول الدهون الثلاثية أظهروا جميعا زيادات معنوية في مستوياتهم قبل عملية غسيل الكلى وينخفض مستوياتهم بعد وأما بالنسبة البروتين الدهني العالي الكثافة فقد أظهرت الدراسة انخفاضا ملحوظا في مستوياتها قبل الغسل وازدادت بشكل ملحوظ بعد الغسل ولكنها بقيت اقل من الحد الطبيعي. كما أظهرت الدراسة الحالية علاقة عكسية بين الباروكسينيز والعمر ومؤشر كتلة الجسم ومع مدة المرض واليوريا والكرياتينين والكولسترول والدهون الثلاثية والبروتين الدهني الواطئ الكثافة والبروتين الدهني الواطئ الكثافة جدا والبروتين الدهني العالي الكثافة.

الاستنتاجات: انخفاض فعالية أنزيم الباروكسينيز في المرضى المصابين بالعجز الكلوي المزمن بشكل معنوي قبل الغسل وزيادته بعد الغسل وبقائه اقل من الحد الطبيعي له ووجود علاقة سلبية بين هذا الأنزيم وبين العمر ومؤشر كتلة الجسم ومدة المرض واليوريا والكرياتينين والكولسترول الدهون الثلاثية والبروتين الدهني الواطئ الكثافة والبروتين الدهني الواطئ الكثافة جدا وحامض اليوريك وجود علاقة ترابط ايجابي ما بين والبروتين الدهني العالي الكثافة. وتوضح هذه الدراسة أن هناك زيادة خطر حدوث مضاعفات القلب والأوعية الدموية في المرضى المصابين بالعجز الكلوي المزمن والذين يخضعون لغسيل الكلى عن طريق زيادة الكولسترول والدهون الثلاثية والبروتين الدهني الواطئ الكثافة والبروتين الدهني الواطئ الكثافة جدا و انخفاض البروتين الدهني العالي الكثافة.