Biochemical Changes in Chronic Renal Failure Pre and Post Hemodialysis

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Abstract: The aim of the study was to evaluate Paraoxonase-1 (PON-1), resistin and bone mineral markers (Parathyroid Hormone (PTH), Ca^{++} and P) levels as biochemical marker for patients with Chronicrenal Failure (CRF). The sample of this study consists of 100 patients and 100 apparently healthy individuals. All patients were those who were receiving Hemodialysis (HD) therapy in the dialysis unit in Kirkuk general. The levels of PON-1, Ca^{++} were significantly lower in CRF patients pre HD when compared with healthy controls, and significantly increased in post HD as compared to pre HD, but still lower than normal controls. Serum P, resistin and PTH levels were significantly higher in CRF patients pre HD process when compared with healthy controls, and their levels were significantly decreased after HD as compared to pre HD, but remained higher than controls. PON-1 was negatively correlated with the resistin, Blood Urea (BU), Creatinine (Cr), PTH, but positively correlated with Ca^{++}. PON-1 activity was significantly decreased in CRF patients before HD and increased after HD, but remained lower than controls. PON-1 was negatively correlated with the Body Max Index (MBI), resistin, BU, Cr, PTH, but significantly and positively correlated with Ca^{++}.

Key words: CRF, HD, PON-1, resistin.

1. Introduction

CRF is characterized by progressive loss of renal functionand decline in estimate glomerular filtration rate [1, 2]. HD is one of the replacement therapy. In this technique, body waste product like urea, Cr and free water are removed from the blood when the kidneys are impaired [3]. CRF is associated with increased levels of some biochemical parameters and decreased others.

2. Material 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 HD therapy in the dialysis unit in Kirkuk public hospital from the period between February 2013 and May 2014.

Exclusion criteria: patients with diabetes mellitus,

schizophrenia, obesity, cancer, Alzheimer's disease, vascular dementia and hepatitis. Blood samples were taken from all cases in the morning between 8-9 a.m. following an overnight fast. The blood samples were centrifuged at 3,500 rpm for 10 minutes, then the serum samples stored at freeze until assayed. Serum resistin and PTH levels were estimated by Enzyme-Linked Immuno Sorbent Assay (ELISA) method. Paraoxonase activity assay was implemented using paraoxon as a substrate conferring to the method described by Mackness, M. [4]. Serum Ca^{++} and P levels were determined by flame-photometry. BU and Cr levels were measured by spectrophotometric kit. For analysis, the height and weight measurement was used to calculate the BMI as a weight in kilograms divided by height in meter squared (m^2).

All results are presented as mean ± SD. Student's t-test was used for the analysis of data. Correlation coefficient used to find the correlation between studied markers by using Pearson correlation.

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3. Results

The baseline and demographic characteristics of the subjects of the study population are shown in Table 1. No significant difference was observed between the cases and the controls with respect to their ages and BMI. The average duration of HD therapy for the CRF patients in the present study was 2.19 ± 0.76 years.

In the present study, blood urea and serum creatinine showed a significant increase in their levels in patients with CRF before when compared to those of controls. However, the serum PON-1 activity was found to be significantly reduced in patients with CRF before HD than in the controls. After HD, urea and creatinine levels remained at high levels, whereas serum PON-1 activity remained low when compared with control groups.

In the current study, before HD, there was a significant rise in the serum resistin 5 ± 0.39 ng/mL when compared with apparently healthy control values of 3.43 ± 0.26 ng/mL. After HD samples, mean values of resistin (4.14 ± 0.15 ng/mL) fail clearly but still higher than the apparently healthy control values (3.43

± 0.25 ng/mL) as depicted in Table 2.

This study detected that the serum calcium level was significantly decreased in patient with CRF (1.83 ± 0.09 mmol/L) before HD when compared with the apparently healthy controls (2.33 ± 0.13 mmol/L) and its level increased after HD (2.00 ± 0.13 mmol/L), but it remained lower than the controls. Before HD, the serum PTH and P were 41.36 ± 9.06 pg/mL and 2.20 ± 0.30 mmol/L, respectively, which were significantly higher than the apparently healthy controls of PTH (19.32 ± 3.63 pg/mL) and 1.04 ± 0.15 mmol/L of P. After HD, both of them showed significantly decreased in their levels, PTH was 29.40 ± 8.45 pg/mL and P was 1.89 ± 0.36 mmol/L, which were also higher than the apparently healthy control in CRF patients.

Table 3 represents that the levels of urea and Cr significant reduced after the HD were 22.96 ± 5.70 mmol/L and 0.69 ± 0.16 mmol/L, respectively. Compared to before HD (29.40 ± 6.40) mmol/L and (0.90 ± 0.15) mmol/L, it remained higher than the healthy controls level serum. PON-1 activity also showed a significantly increased after HD (90.62

Table 1 Demographic characteristics of study population.

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

[*]$P < 0.05$, [**]$P < 0.01$.

Table 2 Biochemical parameters in patients with CRF before and after HD and in apparently healthy controls.

Biochemicalparameters	CRF patients before HD (mean ± SD)	CRF patients after HD (mean ± SD)	Apparently healthy controls (mean ± SD)
BU (mmol/L)	29.40 ± 6.40[*]	22.96 ± 5.70[*]	4.53 ± 0.816
Cr (mmol/L)	0.90 ± 0.15[*]	0.69 ± 0.16[*]	0.08 ± 0.01
PON1 (IU/L)	50.02 ± 6.63[*]	90.62 ± 9.14[*]	149.41 ± 6.19
Resistin (ng/mL)	5± 0.39[*]	4.14± 0.15[*]	3.43 ± 0.25[*]
Parathyroid hormone (pg/mL)	41.36 ± 9.06[*]	29.40 ± 8.45[*]	19.32 ± 3.63[*]
Calcium (mmol/L)	1.83 ± 0.09[*]	2.00 ± 0.13[*]	2.33 ± 0.13 [*]
Phosphorus (mmol/L)	2.20 ± 0.30[*]	1.89 ± 0.36[*]	1.04 ± 0.15[*]

[*]$P < 0.05$, [**]$P < 0.01$.

Table 3   Biochemical parameters in patients with CRF before and after hemodialysis.

Biochemical parameters	CRF patients before HD (mean ± SD)	CRF patients after HD (mean ± SD)
Urea (mmol/L)	29.40 ± 6.40*	22.96 ± 5.70*
Creatinine (mmol/L)	0.89 ± 5.31*	0.68 ± 16.30*
Paraoxonase-1 (IU/L)	50.02 ± 6.63*	90.62 ± 9.14*
Resistin (ng/mL)	5 ± 0.39*	4.14 ± 0.15*
Parathyroid hormone (pg/mL)	41.36 ± 9.06*	29.40 ± 8.45*
Calcium (mmol/L)	1.83 ± 0.09*	2.00 ± 0.13*
Phosphorus (mmol/L)	2.20 ± 0.30*	1.89 ± 0.36*

*$P < 0.05$, **$P < 0.01$.

Table 4   Correlation of PON-1 to other parameters.

Parameters	Pre HD (r)	P	Post HD (r)	P
Resistin (ng/mL)	-0.348*	0.000	-0.272*	0.007
Urea (mmol/L)	-0.477*	0.000	-0.831*	0.000
Creatinine (mmol/L)	-0.843*	0.000	-0.783*	0.000
Calcium (mmol/L)	0.336*	0.001	0.350*	0.000
Phosphorus (mmol/L)	-0.234*	0.019	-0.295*	0.003

± 9.14) IU/L as compared to before HD (50.02 ± 6.63) IU/L. There was a significant decreased in mean values of resistin (4.14 ± 0.15) ng/mL after HD samples in CRF patients as compare to mean values of samples before HD (5 ± 0.39) ng/mL ($P < 0.05$).

There were highly significant differences between the levels of serum Ca^{++}, P and PTH after HD process, the serum Ca was 2.00 ± 0.13 mmol/L which was significantly higher than its concentration in pre HD (1.83 ± 0.09 mmol/L). The mean of serum PTH showed a significantly decreased in its level in post HD stage (29.40 ± 8.45 pg/mL) as compared with its concentration before HD (41.36 ± 9.06 pg/mL). The level of phosphorus also showed a significantly decreased after HD (1.89 ± 0.36 mmol/L) when compared with its concentration in pre HD (2.20 ± 0.30 mmol/L) in patients of CRF disease.

The results presented that the correlation coefficients between serum PON-1 with resistin and the serum bone mineral markers (Ca^{++}, P and PTH) in CRF patients pre and post HD. There was a strong significant positive correlation between PON-1 and Ca^{++} in both pre (r = 0.336) and post (r = 0.350) HD. Phosphorus showed a significant negative correlation with PON-1 in pre (r = -0.234) and post (r = -0.295) HD. There was a significant negative correlation

between PON-1 and PTH in both pre (r = -0.214) and post (r = -0.231) HD. Resistin showed a strong negative correlation with PON-1 in pre HD (r = -0.348) and significantly negative in post HD (r = -0.272) as depicted in Table 4.

4. Discussion

Paraoxonase is an enzyme associated with HDL. It has been shown to prevent atherosclerosis by inhibiting oxidation of LDL. Cardiovascular disease is the main cause of mortality and morbidity in patients with CRF and undergoing HD. In this study, the mean activity of PON-1 was lower in patients with CRF before and after HD as compared to apparently healthy controls. Increase in the activities of PON-1 have been evident during post HD as compared to pre HD samples of patients with CRF. The mean activity of PON-1 were decreased before hemodialysis samples of patients with CRF as compared to apparently healthy controls. PON-1 activity was increased in CRF patients during post HD as compared to pre HD sessions but its concentration is still lower than the normal healthy controls level. This is in accordance with the study of Nagane, N. et al. [5] and Gugliuccil, A. [6]. HD 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 (AGE) products 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. During HD procedure, AGE free adducts may be removed, along with the uremic toxins urea and Cr. Thus, the inhibition of PON-1 may be removed.

Resistin is a protein mediator secreted by adipocytes and macrophages within the adipose tissue. Inhumans, resistin is essentially expressed in macrophages from white adipose tissue, rather than in human adipocytes [7]. Plasma resistin level has recently been shown to be associated with markers of Chronic Kidney Disease (CKD), and it is speculated that inflammatory, metabolicand vascular abnormalities associated with increased circulating resistin levels may have a pathogenic role in CKD [8]. The close relationship between GFR and plasma resistin level favors the possibility of reduced filtration of resistin with declining GFR. Nusken, K. D. et al. [9] suggested that renal function is an important factor in the regulation of the systemic levels of resistin. Risch, L. et al. [10] failed, however, to demonstrate an association between GFR and serum resistin at GFR > 60 mL/min/1.73 m^2, suggesting that resistin level in mildly impaired and normal renal function is influenced by factors other than GFR. In the present study, there was a significant increase in the serum level of resistin when compared with their normal values. In post HD samples, the mean values of resistin fail clearly but still higher than the normal control values. These results are compatible with the studies of Axelsson, J. et al., Subhashini, Y., and Kielstein Jan, T. et al. [11-13]. HD does not eliminate resistin as expected for its molecularsize of 12.5 kDa [14].

Chronic renal failure is a gradual, progressive and irreversible loss of normal functioning of kidneys. As the excretory function of kidney is impaired, urea and Cr excretion is hampered leading to its increased levels in blood, so significant elevation in blood urea and serum creatinine levels are observed in CRF patients before HD. In the present study, the serum urea and creatinine levels were significantly high in pre HD and control groups and was significantly reduced after HD. These results are compatibles with the previous studies [15, 16] which explained that the continued decreased in renal clearance or glomerular filtration rate, leads to the gathering of urea, creatinine and other chemicals in the bloodand CRF which applies to the process of containing significant irreversible reduction in the nephron. In CRF, the increase of serum urea is proportional to the progression of the disease, but it is highly influenced by a catabolic state or an excessive protein ingestion, leading to a higher production of other waste substances of protein catabolism [17]. In end-stage kidney disease, HD treatment is applied to remove excess fluid and waste products, such as urea and Cr from the blood. In this thesis, these parameters show a significant decrease in theirlevels in the post HD samples for both BU and serum Cr, but their concentrations were at a higher range than the normal healthy controls levels. These results are in accordance with the study of Baria, D. et al. [18]. These reductions in mean levels of urea and creatinine are observed in the post hemodialysis treatment. The observation is suggestive of clearance of Cr and urea from blood during HD.

The levels of P and PTH were significantly increased in CRF in pre HD compared with controls, and they were decreased after HD. Inversely, the serum Ca^{++} was significantly decreased in pre HD compared with the controls but its concentration was increased in post HD compared with the pre HD.

These results in the present study are in accordance with the study of Shahbazi, N. et al. [19], Kim, G. H., et al. [20], Collinson, A. et al. [21], and Fouque, D. et al. [22]. The body needs precise regulation of minerals such as phosphorus. Many of organs work together to regulate these levels. The intestines absorb minerals, while the kidneys can excrete them. The parathyroid gland releases parathyroid hormone to regulate phosphorus. The hormone is increased by decreased calcium or increased phosphate. High parathyroid hormone damage the bone and upsets the levels of phosphate and calcium in the bone [23].

Hyperphosphatemia plays a critical role in the development of secondary hyperparathyroidism and renal osteodystrophy in patients with advanced chronic kidney disease as well as patients on dialysis. The usual cause of hyperphosphatemia is a decrease in renal excretion of phosphate. Advanced renal insufficiency reduces excretion sufficiently to increase serum phosphate (PO_4) [24]. Uremic patients develop hyperplasia of the parathyroid glands due to disturbances in the mineral metabolism [25]. Higher serum intact PTH level is associated with the higher likehood of hyperphosphatemia [26]. Kidney failure is the medical conditions that cause low blood calcium levels or increased phosphate levels can lead to secondary hyperparathyroidism [27].

5. Conclusion

PON1 activity was significantly decreased in CRF patients before HD and significantly increased after HD but remained lower than controls. PON1 was negatively correlated with the age, BMI, resistin, Bu, Cr, PTH and P. PON-1 was positively correlated with Ca^{++}. BU and Cr were increased in CRF patients due to the impairment of kidney function and reduced GFR. The results show that serum resistin are associated with renal impairment in CKD. Longitudinal studies carried out in larger patients cohorts would be helpful in investigation if resistin play a harmful role in the development of CKD, and

would enable further understanding of risk factors for CKD progression or its complications (e.g. cardiovascular disease). Hyperphosphatemia, hypocalcemia hyperparathyroidism may have overarching implications on bone and mineral disorders in CRF patients.

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