The Libyan Conference on Chemistry and Its Applications (LCCA 2021)
(15 – 16 December, 2021)

Serum paraoxonase (PON-1) activity and concentration in patients with 4th stage of chronic kidney disease

Mofida Ramadan Makhloof[1]، Dahlia Ibrahim Badran[2]

[1]Department of chemistry, Faculty of science, Benghazi University, Benghazi, Libya.

[2]Department of Biochemistry, Faulty of Medicine, Suez Canal University, Ismailia, Egypt

ARTICLEINFO

Article history:
Received 15 April 2021
Accepted 30 April 2021
Available online 26 June 2022

Keywords:
Atherosclerosis, cardiovascular disease, Chronic kidney disease, Paraoxonase

Corresponding author :
mmakloof@gamail.com

ABSTRACT

Paraoxonase-1 (PON-1) is an enzyme with a glycoprotein structure that depends on calcium and it has cardio protective effects that protect low-density lipoprotein (LDL) and high-density lipoprotein (HDL) from oxidation and therefore retards atherosclerosis. As patients with chronic kidney disease (CKD) are at high risk of atherosclerotic, in this study, we aimed to investigate serum PON-1 activity and concentration levels among patients with 4th stage of CKD compared with the healthy control subjects, to find out the possibility of taking the PON-1 as a predictor marker of atherosclerosis in 4th stage of CKD patients.

Materials and Methods: This study included 30 patients with confirmed diagnosis of 4th stage of CKD, and 30 healthy persons. Serum blood urea, creatinine, uric acid, Albumin, total cholesterol, HDL, LDL and serum triglycerides were measured. Serum level of PON-1 concentration was measured using ELISA technique, while Serum level of PON-1 activity was detected using a colorimetric method.

Results: Our study showed significant increase ($p<0.001$) in the levels of Cholesterol, Triglyceride, LDL and VLDL in patients with 4th stage of CKD as compared to control group. Also, we found significant decreased levels of serum HDL and PON-1 in 4th stage of CKD as compared to control group ($p<0.001$).

Conclusion: The diminution in the serum paraoxonase-1 activity and concentration may contribute to the accelerated development of atherosclerosis in patients with stage 4 of chronic kidney disease.

Introduction

Human serum paraoxonase (PON-1) is a calcium- dependent esterase composed of 354 amino acids (45 kDa) belonging to a family of proteins that includes PON-2 and PON-3 (Primo-Parmo et al., 1996). Genes coding for the PON family are located on human chromosome 7 (q21.22) (Deakin and James, 2004).

PON-1 is synthesized in the liver and secreted into the blood where closely bound to the high-density lipoprotein (HDL) (Blatter et al., 1993), PON-1 has been reported to confer antioxidant activities by decreasing the accumulation of lipid peroxidation products which may prevent the development of atherosclerosis (Mackness et al., 1993).

Oxidation of low -density lipoprotein-cholesterol LDL-c is recognized as the key pathological step in the early development of atherosclerosis leading to uptake of LDL-c by the macrophage scavenger receptor leading to foam-cell formation **(**Witzum,1994). PON-1 is responsible for the breakdown of lipid peroxides before they accumulate on LDL-c, it also inhibits macrophage foam cell formation which contributes to its antiatherogenic property (Rosenblat et al.,2011).

Several studies have demonstrated that serum PON1 activity is inversely associated with oxidative stress (Rozenberg et al.,2003), It has been recently demonstrated that diminished PON-1 activity predicts higher risk of major adverse cardiac events in CKD patients and that low serum concentration of PON-1 may be an independent predictor of cardiovascular disease (CVD) in CKD patients (Gungo et al.,2013).

CVD complications are a major cause of morbidity and early mortality in CKD patient (Collins et al.,2003); therefore, PON-1 may be a clinically useful diagnostic and therapeutic target in the setting of CKD because these patients have limited treatment options that address their significant burden of cardiovascular morbidity and mortality (Kennedy et al. 2013).

Thus, our research aimed to investigate serum PON-1 activity and concentration levels among patients with 4th stage of CKD compared with the healthy control subjects, to find out the possibility of taking the PON-1 as a predictor marker of atherosclerosis in 4th stage of CKD patients.

Materials and Methods

This study was carried out in Internal Medicine department, Suez Canal university Hospital, Ismailia, Egypt, during the Period from 19 March 2019 to 31 September 2019.

Thirty patients in the 4th stage of CKD who were not dialysis (11 women and 19 men) were enrolled in this study, these are with severe reduction in glomerular filtration rate (GFR) (15–29 ml/min/1.73 m ^{2}). The mean age and body mass index (BMI) of this group was 52.37±11.65 years, and 27.30±6.23kg/m^2, respectively.

We also enrolled 30 healthy controls (13 women and 17 men) whose sex, age, and BMI matched with the patients group, healthy controls were selected from regular blood donors in the blood bank. The mean age of healthy controls was 47.60±10.55years and their BMI was 26.27±5.06kg/m^2.

All participants underwent physical examination and echocardiography and each subject provided a detailed medical history. Patients with 4th stage of CKD were diagnosed according to the KDIGO Clinical Practice Guideline for the Evaluation and Management of CKD.

The designated criteria for exclusion included patients with conditions that could affect the PON-1 activity such as Liver diseases, thyroid disorders, alcohol abuse, malignancies, lipid profile altering diseases, Infectious diseases

The body mass index (BMI) was calculated for all participants [BMI= weight (kg)/height (m)^2].

***Blood sampling*:** Blood samples were obtained after overnight fasting, six milliliters of venous blood were collected into sterile vacutainers with gel, left to clot, centrifuged at 1000g for 10 min, then the serum samples were collected and stored at-20^{o}C for further analysis.

***Laboratory investigation*:** All patients and controls were subjected to full laboratory investigations of serum creatinine, urea, uric acid, and albumin. All investigations were performed on Hitachi autoanalyzer (912 Hitachi, Roche, Japan). The colorimetric measurements of total cholesterol, triglyceride, HDL-C in serum were performed using the commercially available kits (SPINREACT, Spain). The low -density lipoprotein-cholesterol (LDL-C) level was calculated using the Friedewald equation [LDL-C= TC – (HDL-C + TG/5)].

The serum concentration PON1 activity was measured using commercially available kits (Relassay ®; Turkey) (Ackerson et al., 1983). PON1 concentration was determined by an in-house enzyme-linked immunosorbent assay (ELISA) (Blatter et al., 1994).

Statistical analysis

In this work, data were collected and entered to the computer using Statistical Package for the Social Science (or SPSS software version 22; SPSS INC., Chicago, USA). The results expressed as Mean ± Standard Deviation (SD) to show the lower and the upper limit. Normality test of the data were tested by using the Shapiro-Wilk W test. Comparison of continuous data between two groups was made by using unpaired t test for parametric data and Mann-Whitney test for nonparametric data. Pearson's correlation coefficient was calculated for correlation between variables. A value of $p<0.05$ was considered to be statistically significant. Receiver operating characteristic curves (ROC curves) were drawn to assess the validity of the biomarkers.

A *P* value < 0.05 was considered significant

Results

Table 1 demonstrates the number, age, sex, BMI, eGFR(ml/min/1.73m^2) and the results of different biochemical parameters carried out in this study including

Parameter	CKD (n=30) Mean ± SD	Control (n=30) Mean± SD	t-value	P
Sex (male/female)	19/11	17/13		
Age (years)	52.37±11.65	47.60±10.55	1.66	>0.05
BMI (kg/m^2)	27.30±6.23	26.27±5.06	0.71	>0.05
eGFR (ml/min/1.73 m^2)	22±40	93±33	-11.01	<0.001
Creatinine (mg/dL)	3.93±1.88	0.56±.21	9.73	<0.001
Urea (mg/dL)	78.93±27.04	27.04±8.10	10.24	<0.001
Albumin (g/dL)	3.26±1.0	4.04±1.10	-2.86	.<0.001
Uric acid (g/dl)	9.69±1.66	5.90±2.02	7.93	<0.001

different kidney function testes of individuals from all investigated groups**.**

Table 1: Demographic and laboratory characteristics in CKD patients and control group.

There was a significant increase in the mean level of serum Creatinine, Urea and Uric acid and significant decrease in the mean level of serum Albumin in patients with 4th stage of CKD group when compared to the control group (P <0.001) (Table 1).

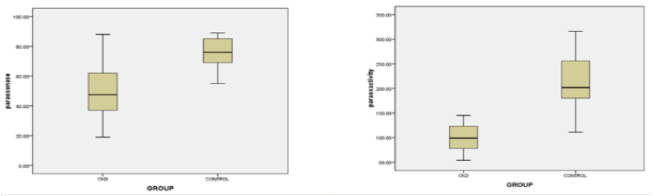
As illustrated in the table2, serum Triglyceride, Cholesterol and LDL-c were significantly higher in the patients with 4th stage of CKD compared to the control group (p <0.001), While a significant decrease in serum HDL-c (p <0.001).

Table 2: The lipid profile, serum PON1 concentration and activity of the CKD and control group.

Parameter	CKD(n=30) Mean ± SD	Control(n=30) Mean ± SD	Test-value	P
Triglyceride (mg/dl)	152.33±55.45	87.87±18.61	6.04	<0.001
Cholesterol (mg/dl)	183.66±49.12	142.60±23.50	4.13	<0.001
HDL-c (mg/dl)	34.16±10.48	43.10±11.33	-3.170	<0.001
LDL-c (mg/dl)	114.55±53.01	84.35±24.17	2.84	<0.001
PON1. conc (g/ml)	48.96±16.40	75.17±9.35	-7.61	<0.001
PON1 activity (U/L)	101.93±27.67	212.80±53.57	-10.07	<0.001

Regarding to the results of mean serum levels of PON1 concentration (ng/ml) level, table 2, Figure1 showed a significant decreased in the patients with 4th stage of CKD group compared to the control group (P< 0.001). The same finding regarding the serum level of PON-1 activity which was significantly decreased in the 4th stage of CKD patients compared to the control group, table 2, Figure 1 (P< 0.001).

Figure1: Box plots showing the level of studied markers in the 4th sta ge of CKD patients and control groups



A:Comparison between PON1 concentration levels in Patients with CKD and control subjects

B:Comparison between PO1 activity levels in patients with CKD and control subjects

Diagnostic Performance of studied markers

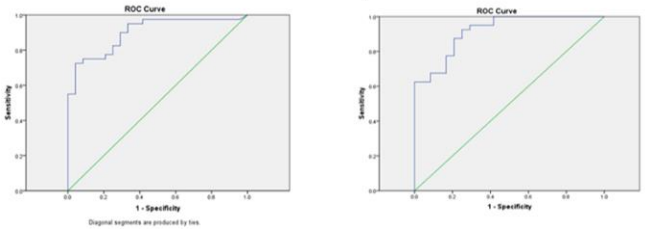
Figure 2 represented the ROC curve of serum PON`1concentration and activity to differentiate CKD patients from healthy subjects, when analysis of the results of the ROC curve, this study found the Area Under the curve (AUC) was 0.92 for serum PON-1 activity (U/L) with sensitivity 86.84%, specificity 73.08%, PPV 81.54%, and NPV 76.15% at optimal cutoff value 109.22ng/ml (table 3).

While the AUC for serum PON1 concentration (ng/ml) was 0.86 with sensitivity 81.82%, specificity 80.00%, PPV 92.00%, and NPV 65.62% and optimal cutoff value 76.51 (ng/ml) (Table 3).

Table 3: Analysis showing Cut-off, Sensitivity, Specificity, AUC, PPV and NPV of the Studied Markers.

Parameter	PON-1 activity (U/L)	PON-1con (ng/ml)
Cut off value	109.22	76.51
Sensitivity (%)	86.84	81.82
Specificity (%)	73.08	80.00
Positive predictive value (%)	81.54	92.00
Negative predictive value (%)	76.15	65.62
Youden's index	0.60	0.62
Area Under the ROC	0.86	0.92
P- value	<0.001	<0.001

Figure 2: the diagnostic performance of the studied markers in CKD patients vs. control subjects.



(A) PON1con: ROC curve **(B) PON1activity: ROC curve**

In the present study, there was significant negative correlation have been found between levels of serum PON-1concentration and PON-1 activity with the studied parameters such creatinine, urea Uric acid, triglyceride, cholesterol, in (Table 4), except for a positive correlation between serumPON-1concentration and PON-1 activity with HDL-**c.**

Table 4: Correlation of PON1 activity and concentration with different variables in CKD group.

Parameter	PON1 activity		PON1 concentration	
	r	p	r	p
Age (years)	0.006	> 0.05	-0.215	> 0.05
BMI (kg/m2)	-0.155	> 0.05	0.014	> 0.05
eGFR(ml/min/1.73m2)	-0.09	> 0.05	-0.01	> 0.05
Creatinine(mg/dL)	-0.660*	< 0.05	-0.534*	< 0.05
Urea (mg/dL)	-.632*	< 0.05	-0.596*	< 0.05
Albumin	0.128	> 0.05	0.283*	< 0.05
Uric acid	-0.500*	< 0.05	-0.549*	< 0.05
Triglyceride (mg/dl)	-0.512*	< 0.05	-0.494*	< 0.05
Cholesterol (mg/dl)	-0.387*	< 0.05	-0.307*	< 0.05
HDL-c (mg/dl)	0.349*	< 0.05	0.423*	< 0.05
LDL-c (mg/dl)	-0.248	> 0.05	-0.23	> 0.05

* Correlation is significant at the 0.01 level (2-tailed). Spearman non-parametric test is used.

P < 0.05 (significant), P > 0.05 (insignificant

Discussion

Chronic kidney disease (CKD) is associated with an increased risk of cardiovascular disease as these patients develop accelerated atherosclerosis (Bikbov. et.al.,2020) The main mechanisms underlying this

increased CV risk in this population are oxidative stress (Vera, et.al., 2018), accumulation of uremic toxins (Fujii et al., 2018), dyslipidemia (Vaziri.,2006).

In the present study blood urea and serum creatinine were significantly elevated in patients with CKD when compared to controls as shown in table 1. This was in agreement with Pandya. D et.al.,2016 and Sridevi et.al.,2020, who report differences in blood urea and serum creatinine in patients with 4th stage of CKD when cumbered to controls subjects.

Moreover, the serum uric acid is significantly increased in CKD patients compared to healthy individuals. Our results were similar to those obtained by Kim et.al.,(2021).

Renal insufficiency combined with heavy proteinuria leads to acquired LDL receptor deficiency which plays a critical role in the origin of hypercholesterolemia associated with chronic kidney disease. In the present, study serum cholesterol, LDL-c and Triglyceride levels were elevated in CKD patients when compared to controls as shown in table 2. while HDL-c showed a significant decrease in comparison with control group. Similar results were found by Wong et al., (2015), Sridevi et.al., (2020) and Kawachi.et.al.,(2019) studies.

PON-1 enzyme has many functions, among which are preventing oxidative modification of low-density lipoprotein (LDL) particles (Précourt et al., 2011).

The results of the present study showed significant lower levels of PON-1 activity among CKD patients compared with the healthy controls (P< 0.001). Our finding is comparable with the previous studies done by Kuchta et al., (2011) and Vasylchenk et al., (2020) who have demonstrated a significant decrease in PON-1 activity in predialysis chronic kidney disease patients, Saeed et al., (2008) have also observed reduced PON-1 activity in CKD patients.

Additionally, we found that serum PON1 activity cut off is at a value 109.22(U/L), sensitivity (86.84%), specificity of (73.08%), PPV was (81.54%), NPV was (76.15%) and AUROC was (. 0.86).

In our study, serum PON- 1concentration was measured by the sandwich ELISA technique in patients with 4th stage of CKD and control, the results in the current study indicate that serum level of PON-1 concentration was significantly decreased in patients with 4th stage of CKD when compared with healthy controls (P< 0.001).

Besides, ROC curve analysis showed optimal cut off value for PON- 1concentration was76.55 while sensitivity (81.82%), specificity (80.00%), PPV (92.00%), NPV (65.62%) and AUROC and (0.86).

Only few studies are available regarding PON-1 concentration in patients with CKD, our results agree with Marsillach et al., (2007) who reported

the concentration of PON1 was significantly decrease in in patients with CKD.

Furthermore, a study by Samouilidou et al., (2016) reported PON1 concentration was decreased in CKD patients compared to controls but this decrease was not statistically significant.

The causes of reduced enzyme activity and concentration are unclear. Experimental studies show that it is possible to inactivate the enzyme by uremic toxins, including reaction products of reactive oxygen species (ROS) (Fujii et al., 2018). The oxidation of LDL-C phospholipids due to oxidative stress conditions inhibit the synthesis of PON-1 in the liver is one of the first steps in the atherosclerotic process (Witztum and Steinberg,1991), (Mackness et al.,1993).

The reduced activity of PON-1 may be the consequence of lower concentrations of HDL-C in CKD because HDL is the leading carrier of PON-1.

Conclusions

Patients with4th stage of CKD had a significant increase in cholesterol, triglyceride and LDL and significant decrease in HDL-c, PON-1concentration and PON-1 activity. Our study concludes that altered serum lipid profile and paraoxonase may be responsible for the higher prevalence tendency for atherosclerosis in these patients.

Estimation of this enzyme activity is a better to predict and prevent cardiovascular complications arising from advanced CKD

However, further studies on a large scale are needed to assess the diagnostic and the prognostic values of serum concentration and activity PON1 in patients with CKD.

References

1-Primo-Parmo SL, Sorenson RC, Teiber J and La Du BN (1996) The human serum paraoxonase/ arylesterase gene (PON1) is one member of a multigene family. Genomics 33:498–507.

2-Deakin SP and James RW (2004). Genetic and environmental factors modulating serum concentrations and activities of the antioxidant enzyme paraoxonase-1. Clin Sci (Lond) 2004; 107: 435–47.

3-Blatter M-C, James RW, Messmer S, et al (1993) Identification of a distinct human high-density lipoprotein subspecies defined by a lipoprotein-associated protein, K-45. Identity of K-45 with paraoxonase. Eur J Biochem;211:871.

4-Mackness MI, Arrol S, Abbott CA, et al.(1993)Is paraoxonase related to atherosclerosis? Chem Biol Interact;87:161.

5-Witzum JL. (1994) The oxidation hypothesis of atherosclerosis. Lancet, 344: 793-795.

6-Rosenblat M, Volkova N, Ward J et al. (2011) Paraoxonase 1 (PON1) inhibits monocyte to macrophage differentiation. Atherosclerosis; 219 (1):49-56.

7-Rozenberg O, Rosenblat M, Coleman R et al. (2003)

Paraoxonase (PON1) deficiency is associated with increased macrophage oxidative stress: studies in PON1-knockout mice. Free Radic Biol Med; 34: 774–784.

8-Collins AJ, Hanson G, Umen A, et al. (1990) Changing risk factor demographics in end-stage renal disease patients entering hemodialysis and the impact on long-term mortality. Am J Kidney Dis;15:422-32.

9-Kennedy JD, Tang W; Fan Y, Yuping Wu, et al (2013) Diminished Antioxidant Activity of High-Density Lipoprotein– Associated Proteins in Chronic Kidney Disease Journal of the American Heart Association, DOI:10.1161/JAHA.13.000104

10-Ackerson LM, Lepper K,Robbins S, Go AS, Yang J,(1983).Associated antioxidant enzyme paraoxonase (PON) 1 and higher concentration of lipid. Biophys Res Commun; 113:666–71.

11-Blatter-Garin MC, Kalix B, De Pree S and James R W (2003). Aspirin use is associated with higher serum concentrations of the anti-oxidant enzyme, paraoxonase-1. Diabetologia 46, 593-594.

12-Gungor O, Kircelli F and TozInt H. (2013) Paraoxonase 1, atherosclerosis and arterial stiffness in renal patients. Urol Nephro,l 45:441–447

13-Bikbov B., Purcell C.A., Levey A.S., Smith M., Abdoli A., Abebe M., Adebayo O.M., Afarideh M., Agarwal S.K., Agudelo-Botero M., et al. (2020; Global),regional, and national burden of chronic kidney disease, 1990–2017: A systematic analysis for the global burden of disease study. *Lancet.* 395:709–733.

18-Vera M., Torramade-Moix S., Martin-Rodriguez S., Cases A., Cruzado J.M., Rivera J., Escolar G., Palomo M., Diaz-Ricart M. (2018). Antioxidant and anti-inflammatory strategies based on the potentiation of glutathione peroxidase activity prevent endothelial dysfunction in chronic kidney disease. *Cell. Physiol. Biochem.* ;51:1287–1300.

19-Vaziri ND. (2006) Dyslipidemia of chronic renal failure: the nature, mechanisms, and potential consequences. Am J Physiol Renal Physiol;290(2):F262-72.

20-Fujii H, Goto S and Fukagawa M. (2018), Role of Uremic Toxins for Kidney, Cardiovascular, and Bone Dysfunction. Toxins, 10, 202.

21-Kim IY, Ye BM, Kim MJ, Kim SR, Lee DW, Kim HJ, et al. (2021) Association between serum uric acid and left ventricular hypertrophy/left ventricular diastolic dysfunction in patients with chronic kidney disease. PLoS ONE 16(5): e0251333.

22-Pandya, D., Nagrajappa, A. K., Ravi, K. S (2016) Assessment and Correlation of Urea and Creatinine Levels in Saliva and Serum of Patients with Chronic Kidney Disease, Diabetes and Hypertension- A Research Study. Journal of clinical and diagnostic research: JCDR; 10(10): ZC58–ZC62.

23-Sridevi C, Sowjanya U, Selvi V, Rajakumari4 D and Babu5 K. (2020) Serum Paraoxonase with HDL-C as a predictor of atherosclerosis in patients of Chronic Kidney Disease Biomedicine; 40(4): 442-446.

24-Kawachi, K., Kataoka, H., Manabe, S., Mochizuki, T., Nitta, K. (2019) Low HDL cholesterol as a predictor of chronic kidney disease progression: a cross-classification approach and matched cohort analysis. Heart Vessels. Sep;34(9):1440-1455.

25-Wong, M. G., Wanner, C., Knight, J., Perkovic, V (2015) Lowering cholesterol in chronic kidney disease: is it safe and effective?. European Heart Journal; 36(43); 2988–2995.

26-Precourt, L. P., Amre, D., Denis, M. C., Lavoie, J. C., Delvin, E., Seidman, E., and Levy, E. (2011). The three-gene paraoxonase family: physiologic roles, actions and regulation. Atherosclerosis 214, 20-36.

27-Kuchta A, Pacanis A, Kortas-Stempak B et al (2011) Estimation of oxidative stress markers in chronic kidney disease. Kidney Blood Press Res 34:12–19

28-Saeed SA, Elsharkawy M, Elsaeed K, Fooda O (2008) Paraoxonase-1 (PON1) activity as a risk factor for atherosclerosisin chronic renal failure patients. Hemodial Int 12:471–479.

29-Vasylchenk O, Korol L, chmenk Oand StepanovaN (2020,)The oxidative status in patients with chronic kidney disease ISSN 2409-4943. Ukr. Biochem. J., Vol. 92, N 5.

30-Marsillach J, Martinez-Vea A, Marcas L et al (2007) Administration of exogenous erythropoietin b affects lipid peroxidation and serum paraoxonase-1 activity and concentration in predialysis patients with chronic renal disease and anemia. Clin Exp Pharmacol Physiol 34:347–349.

31-Samouilidou E, Kostopoulos V, Liaouri A, Kioussi E, Vassiliou K, Bountou E and Grapsa E (2016) Association of lipid profile with serum PON1 concentration in patients with chronic kidney disease. *Renal Failure*, VOL. 38, NO. 10, 1601–1606.

32-Mackness MI, Arrol S, Abbott C, Durrington PN. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. Atherosclerosis 1993;104:129–35.

33-Witztum JL, Steinberg D. Role of oxidized low-density lipoprotein in atherogenesis.
J Clin Invest 1991;88:1785–92.