Serum Paraoxonase 1 and Gene Polymorphism (PON- Q192R) as an Atherosclerotic Marker in Chronic Kidney Disease Patients in Menoufia University Hospitals- Egypt

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ABSTRACT

Background: Patients with chronic kidney disease (CKD) or end stage renal disease (ESRD) are at increased risk of atherosclerosis and cardiovascular event. Paraoxonase 1 has atheroprotective effects through its role in lipid peroxidation.

Objectives: This study aimed to check serum paraoxonase 1 level and gene polymorphism (PON 1- Q192R) as an atherosclerotic marker in CKD patients in Menoufia University Hospitals, Egypt.

Patients and methods: This case-control study included 65 patients with CKD attending Menoufia University Hospitals. Patients were classified into 2 groups: 30 patients on conservative therapy (Group II) and 35 ESRD patients on regular hemodialysis (HD) more than 6 months (Group III). Thirty healthy age- and gender-matched individuals were evaluated as control group (group I). Complete blood count, lipid profile, blood urea, serum creatinine, C-reactive protein, serum homocysteine and PON1 lactonase activity (human serum paraoxonase 1) were measured using commercially available enzyme-linked immunosorbent assay kits and genetic study (PON 1-Q192R) was determined by Real time polymerase chain reaction (PCR). Carotid intima media thickness (CIMT) was measured through ultrasonographic examination of carotid arteries.

Results: CIMT was significantly higher in CKD patients than in control group. Serum PON 1 was significantly lower in ESRD patients on HD than in CKD patients predialysis, which in turn was significantly lower than in healthy control subject. There was statistical significant negative correlation between CIMT and PON 1 level. There was statistically significant difference between patients and control as regards paraoxonase gene polymorphism. Genotype QQ and Q allele were common in CKD & ESRD patients than in control.

Conclusion: Serum paraoxonase 1 level may be a potential biomarker of atherosclerosis in CKD patients.

Keywords: CKD, ESRD, Atherosclerosis, CIMT, paraoxonase 1.

INTRODUCTION

Oxidative stress is the main cause of atherosclerosis, which is characterized by the accumulation of fatty lesions, inflammation, and scarring of artery walls. Atherosclerotic lesions may arise mostly from oxidative alterations of low density lipoproteins (LDL) in the artery wall. It's established that oxidative stress promotes the production of oxidized LDL [(1)].

Atherosclerosis is more common in patients with chronic renal failure (CRF) of several causes [(2)]. For patients on ESRD, cardiovascular events constitute the leading cause of death [(3)]. Oxidative stress is one of the two known risk factors for CVD [(4)]. Hypertriglyceridemia, elevated remnant particles, low quantities of HDL cholesterol, and other lipoprotein abnormalities are common in patients with CRF. There is an inverse relationship between atherogenic risk and HDL cholesterol levels [(5)].

PON1, PON2, and PON3 are the three members of the paraoxonases (PONs) enzyme family. The structural arrangement of proteins is comparable among these genes, which are substantially identical. Two additional members of the PON gene family are generated by the liver and interact with high density lipoprotein (HDL), whereas PON2 is an intracellular enzyme that is expressed in a variety of tissues and organs [(6)].

Paraoxonases are involved in various biochemical pathways, such as protecting the body from oxidative damage and lipid peroxidation, enhancing innate immunity, eliminating reactive molecules from the body, triggering drug bioactivation, managing endoplasmic reticulum stress, and controlling cell division and apoptosis [(7)]. PONs have the ability to hydrolyze and hence detoxify homocysteine thiolactone and oxidized LDL, two highly proatherogenic and cytotoxic substances. PONs do, in fact, have a multitude of atheroprotective qualities, such as anti-apoptotic, anti-thrombosis, anti-adhesion, antioxidant activity, anti-inflammatory action, preservation of HDL function, and promotion of cholesterol efflux [(6)].

Low blood concentration of PON1 may be an independent predictor of cardiovascular mortality in hemodialysis patients, and it has recently been shown that reduced PON1 activity predicts increased risk of significant adverse cardiac events in CKD patients [(8)].

PATIENTS AND METHODS

This case-control study was conducted on 65 patients with CKD selected from Outpatient Clinics, Inpatient Wards and Haemodialysis Unit at Internal Medicine Department of Menoufia University Hospitals, Egypt. 30 healthy age- and gender-matched individuals served as control from July 2022 to July 2023.

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The study participants were classified into 3 groups: **Group I (control group) (N=30)** included apparently healthy normal individuals, **group II (N=30)** included CKD patients on conservative therapy - Predialysis group), and **group III (N=35)** that included CKD patients on regular HD (ESRD), 3 sessions/week for more than 6 months.

Inclusion criteria: All adult patients more than 18 years' age of both sexes and patients with CKD stages 2- 5 were included in this study.

Exclusion criteria: Patients with decompensated liver disease or elevated liver enzymes, patients with recent myocardial infarct, pregnancy, lactation, patients on lipid-lowering therapy, estrogen therapy, oral antidiabetics or antioxidants, smokers, patients with hyperthyroidism, hypothyroidism and subclinical forms of thyroid diseases and alcoholic patients.

All the studied patients and 30 healthy control subjects were subjected to the following: Thorough history taking and complete clinical examination and anthropometric measurements (BMI was calculated as body weight (in kg) divided by height (in squared meters) (kg/m^2).

Laboratory investigations: - routine investigations: CBC, kidney function tests (blood urea &serum creatinine), data on mineral bone disease (corrected serum calcium, phosphorus & intact PTH), estimated GFR was calculated using CKD-EPI equation, estimation of lipid profile (including TC, TG, HDL, and LDL), liver function tests (T. bilirubin, S. albumin, ALT & AST). Special investigations included serum hsCRP that was measured by ELISA using Kits by Turbidimetry and serum total homocysteine concentrations were determined by ELISA Kits. Serum PON1 lactonase activity (human serum paraoxonase 1) was measured by a commercially available ELISA Kits. Paraoxonase 1 gene polymorphism assay: Genomic DNA was isolated from venous blood, and the Q192R variation of the PON1 gene (SNP ID: rs662) was discovered by real-time PCR.

CIMT: Genomic DNA was extracted from venous blood and the Q192R variant of the PON1 gene (SNP ID: rs662) was found by real-time PCR. Echocardiography: for left ventricular diameter (concentric left ventricular hypertrophy) & wall motion abnormalities.

Methods of sampling: Each individual had a venipuncture to obtain 5 ml venous blood sample under strict aseptic conditions. After allowing the samples to coagulate for ten to twenty minutes at room temperature, the supernatant was collected by centrifuging the samples for twenty minutes at a speed of 2000 to 3,000 rpm. Before testing, specimens were frozen for a longer period of time—only once, at -40°C.

-**Assessment of serum paraoxonase 1:** Assay principle: To measure the amount of human paraoxonase 1 (PON) in samples, the kit was built on a double-antibody sandwich ELISA (Sunred Biotechnology Company, Shanghai, China). Add PON to the well-coated monoclonal antibody enzyme and let it incubate. Next, add PON antibodies that have been biotin-labeled and mix them with streptavidin-HRP to create an immunological complex. Finally, repeat the incubation and washing steps to get rid of the uncombined enzyme. After adding Chromogen Solution A and B, the liquid's color shifts to blue, and eventually becomes yellow due to the action of sulfuric acid. At a wavelength of 450 nm, the chroma of color was determined spectrophotometrically. The O.D. of the samples was then compared to the standard curve to determine the concentration of human PON 1 in the samples.

- **Paraoxonase 1 gene polymorphism assay:**

Using Real-time PCR (TaqMan ® Allelic Discrimination test), the Q192R variation of the PON1 gene (SNP ID: rs662) was identified after genomic DNA was isolated from venous blood.

Taqman genotyping assay kit containing both primers and probes (Applied Biosystem, Foster city, USA, 2010) as follows:

- **PRIMER**: Forward primer (5′-GGACCTGAGCACTTTTATGGCA -3′) and Reverse primer (5′-GACAACATACGACCACGCTAAACC-3′)
- **PROBES:** were described by manufacturer as the following: [VIC/FAM]: (TAAACCCAAATACATCTCCCAGGAT**[C/T]**GTAAGTAGGGGTCAAGAAAATAGTG)

(192Q: 5′-FAM-TTCTTGACCCCTACTTACAATCCTGGGAGATGT-3′ and 192R: 5′-VIC-CTTGACCCCTACTTACGATCCTGGGAGATGT-3′.)

During PCR, each allele-specific probe anneals selectively to its complementary sequence and has a reporter dye at its 5'end and a non-fluorescent quencher at its 3'end. The primers attached to the genomic DNA template are extended by Taq polymerase. Probes that have hybridized to the target sequence are broken apart by Taq polymerase. The quencher dye and the reporter dye are separated by cleavage of the probes hybridized to the target sequence, increasing the reporter's fluorescence. The alleles that are present are indicated by the fluorescence produced by PCR amplification.

- **Measurement of CIMT:** IMT definition – The distance between the lumen-intima and media-adventitia is used to calculate IMT. A higher risk of CVD and atherosclerosis is most likely to be indicated by an IMT larger than 0.9–1 mm. Common CIMT was

assessed using a linear (superficial) duplex probe and B-mode ultrasonography using a SonoScape A5 instrument (Guangdong, China). The probe was positioned anterolaterally, and the patients were instructed to lie down in a supine position with their heads slightly angled toward the side that was being studied. Ten millimeters below the carotid bifurcation, on both the left and right common carotid arteries' posterior walls, was where the thickness of the intima-media was measured. Two roughly parallel echogenic lines were seen in longitudinal images of the typical carotid wall's layers, and they were divided by a hypoechoic to anechoic zone. The lumen-intimal interface was represented by the first echo, which is near to the vessel lumen, while the media-adventitia contact is responsible for the second echo. The anechoic/hypoechoic area in between the echogenic lines is known as the media. The total thickness of the media and intima is represented by the distance between these two lines. The total thickness of the media and intima is represented by the distance between these two lines [(9)].

Ethical approval: This study was approved by Menoufia Faculty of Medicine's Medical Ethics Committee. Following receipt of all information, signed consent was provided by each participant. The Helsinki Declaration was adhered to at every stage of the investigation.

Statistical analysis:

For data analysis, SPSS Version 20.0 was employed. The three groups' findings were compared using one-way ANOVA and a post hoc Tukey test after the continuous quantitative variables were provided as mean ± SD. After the qualitative variables were represented as numbers and percentages, they were examined using Fisher's exact test and the χ^2-test. The relationships between quantitative variables were evaluated using Pearson correlation (r), and among probable risk factors, binary regression analysis was done to identify a viable predictor for vascular and valvular calcification. The cutoff level of paraoxonase 1, sensitivity, and specificity for the identification of atherosclerosis in patients with CKD were estimated using the ROC curve. A significant p-value was defined as ≤ 0.05.

RESULTS

In the current study, the mean age of CKD-predialysis group was 56.5 ± 13.2 and of CKD on hemodialysis was 50.1 ± 16.2 years. Most patients in predialysis group were females (66.7 %) but most of CKD on hemodialysis were males (53.3 %). Regarding age and gender, there was no statistically significant difference between the three groups under investigation. The research groups differed significantly in terms of height, weight, and BMI (Table 1).

Table (1): Demographic & anthropometric characteristics of studied groups

	Group (1) (no=30)		Group (2) (no=30)		Group (3) (no=35)		ANOVA Test	P value	Post Hoc Test
	Mean±SD		Mean±SD		Mean±SD				
Age (years)	51.8 ± 12.2		56.5 ± 13.2		50.1 ± 16.2		1.9	0.1	
Height (cm)	169.4 ± 7.3		170.5 ± 8.6		159.8 ± 16.8		7.9	0.001*	
Weight (Kg)	78.8 ± 11.2		81.5 ± 13.4		69.1± 21.1		5.3	0.006*	
BMI(kg/m^2)	23.01 ± 2.6		23.9 ± 3.7		26.4 ± 4.9		6.6	0.002**	P1=0.9 P2 = 0.001** P3= 0.002**
Gender	**No**	**%**	**No**	**%**	**No**	**%**	**X^2 test**	**P value**	
Male	16	53.3 %	10	33.3%	20	57.1 %	4.1	0.1	
Female	14	46.7 %	20	66.7%	15	42.9 %			

P1, comparison between control and CKD predialysis groups; P2, comparison between control and CKD on HD groups; P3, comparison between CKD predialysis and CKD on HD group *Significant; **Highly significant.

There was statistically significant difference between the three studied groups as regards hemoglobin, triglycerides & HDL and highly significant difference as regard (Hb, LDL, calcium, phosphorus, creatinine, e-GFR, i PTH, CRP CRP, homocysteine, CIMT, LVH, and paraoxonase 1 level. But, there was no statistically significant difference between study groups regarding serum cholesterol. The mean value of serum paraoxonase 1 level (PON 1 activity) was lower in CKD and ESRD than in controls and also it was lower in ESRD on HD than CKD patients (Table 2).

Table (2): Comparison between studied groups regarding laboratory investigations

	Group (1) (no=30)	Group (2) (no=30)	Group (3) (no=35)	Anova Test	P value	Post Hoc Test
	Mean±SD	Mean±SD	Mean±SD			
Hb (gm/dl)	13.1 ± 0.5	10.1 ± 1.4	10.12 ± 1.5	62.1	<0.001**	P1= 0.001** P2 = 0.001** P3=0.8
Cholesterol (TC) (mg/dl)	164.7 ± 40.8	186.9 ± 40.9	213.6 ± 51.6	0.6	0.5	
Triglycerides (TGs) (mg/dl)	112.6 ± 27.9	122.9 ± 28.7	140.4 ± 32.9	3.8	0.02*	P1= 0.03* P2= 0.009** P3=0.04*
LDL-c (mg/dl)	79.3 ± 15.1	99.5 ± 14.4	121.9 ± 26.9	17.9	<0.001**	P1= 0.001** P2 = 0.001** P3=0.05*
HDL-c(mg/dl)	43.7 ± 10.2	38.1 ± 9.5	35.1 ± 7.04	3.2	0.04*	P1= 0.03* P2 = 0.02* P3= 0.05*
Calcium (mg/dl)	9.5 ± 0.5	8.02 ± 0.6	8.6 ± 0.8	39.3	<0.001**	P1= 0.001** P2 = 0.001** P3=0.06
Phosphorus (mg/dl)	3.1 ± 0.4	4.9 ± 1.1	5.1 ± 1.2	23.6	<0.001**	P1= 0.001** P2 = 0.001** P3=0.04*
i PTH (pg/dl)	32.1 ± 7.6	304.4 ± 75.3	540.3 ± 133.6	99.4	<0.001**	P1= 0.001** P2 = 0.001** P3=0.02*
Creatinine (mg/dl)	0.7 ± 0.1	2.3 ± 0.5	6.7 ± 1.1	100.1	<0.001**	P1= 0.001** P2 = 0.001** P3=0.001**
CRP (mg/dl)	5 ± 0.8	17.1 ± 4.1	29.8 ± 7.3	10.2	<0.001**	P1= 0.001** P2 = 0.001** P3=0.05*
e-GFR (ml/min/1.73 m^2)	110.01 ± 14.7	23.5 ± 5.6	8.3 ± 1.9	98.5	<0.001**	P1= 0.001** P2 = 0.001** P3=0.000**
Paraoxonase 1 (ng/ml)	78.3 ± 18.9	60.5 ± 14.8	29.7 ± 7.3	22.4	<0.001**	P1 = 0.05* P2=0.001** P3= 0.001**
Homocysteine (nmol/ml)	4.7 ± 1.12	5.9 ± 1.3	10.7 ± 2.4	29.1	<0.001**	P1 = 0.02* P2=0.001** P3= 0.001**
CIMT(mm)	0.5 ± 0.07	1.02 ± 0.2	1.2 ± 0.3	71.1	<0.001**	P1= 0.001** P2= 0.001** P3= 0.002**
LVH by Echo	**No %** 0 0%	**No %** 13 43.33%	**No %** 22 62.86%	**X^2 test** 94 .1	<0.001**	P1= 0.001** P2=0.001** P3= 0.08

P1, comparison between control and CKD predialysis groups; P2, comparison between control and CKD on HD groups; P3, comparison between CKD predialysis and CKD on HD group *Significant; **Highly significant

There was no significant difference among the three studied groups as regards paraoxonase gene polymorphism distribution according to Hardy–Weinberg equilibrium (Table 3).

Table (3): Paraoxonase gene polymorphism (Q192R) distribution according to Hardy–Weinberg equilibrium among studied groups

	Observed		Expected		X^2 test	P value
Group 1 (n=30)	**No**	**%**	**No**	**%**		
QQ (AA, CC)	4	13.3%	2	6.7%	1.6	0.4
QR (AG, CT)	7	23.3%	11	36.7%		
RR (GG, TT)	19	63.4	17	56.6%		
Group 2 (n=30)						
QQ (AA)	14	46.6%	15	50%	0.07	0.9
QR (AG)	8	26.7%	8	26.7%		
RR (GG)	8	26.7%	7	23.3%		
Group 3 (n=35)						
QQ (AA)	17	48.6%	17	48.6%	0.06	0.9
QR (AG)	9	25.7%	10	28.5%		
RR (GG)	9	25.7%	8	22.9%		

There was statistically significant difference between the studied groups as regards genotype distribution, allele frequency. Genotype QQ and allele Q were common in CKD and ESRD on HD than in control group (Table 4).

Table (4): Distribution of genotype and allele frequencies of the PON1 Q192R polymorphisms among studied groups

	Group 1 (N=30)		Group 2 (N=30)		OR	CI 95 %	P value
Variables	**N**	**(%)**	**N**	**(%)**			
QQ (AA, CC)	4	13.3%	14	46.6%	4.1	1.39 to 12.2	0.01**
QR (AG, CT)	7	23.3%	8	26.7%	2.3	0.62 to 8.9	0.2
RR (GG, TT)	19	63.4%	8	26.7%			
Allele							
Q (A, C)	15	25%	30	20.3%	0.3	0.15 to 0.72	0.005**
R (G, T)	45	75%	30	79.7%			
	Group 1 (N=30)		**Group 3 (N = 35)**		**OR**	**CI 95 %**	**P value**
Variables	**N**	**(%)**	**N**	**(%)**			
QQ	4	13.3%	17	48.6%	3.1	1.01 to 9.09	0.03*
QR	7	23.3%	9	25.7%	2.2	0.61 to 8.2	0.2
RR	19	63.4%	9	25.7%			
Allele							
Q	15	25%	35	50%	0.33	0.15 to 0.7	0.004**
R	45	75%	35	50%			

*Significant; **Highly significant; - Q (A, C) allele is the diseased allele.

There was no significant difference between paraoxonase gene polymorphism and the laboratory data (Table 5).

Table (5): Comparison between demographic data, laboratory variables and CIMT of all studied CKD patients (group 2 predialysis and group 3 on HD) with distribution of genotype of the PON1 Q192R polymorphisms (n=65)

Variables	QQ (n=31)	QR (n=17)	RR (n=17)	T Test	P value
	Mean± SD	Mean± SD	Mean± SD		
BMI (kg/m^2)	24.9 ± 4.2	26.1 ± 4.4	25.3 ± 5.4	0.3	0.6
Hb (g/dl)	9.7 ±1.3	10.1 ± 1.9	10.6 ± 1.1	2.1	0.1
CRP (mg/dl)	24.4 ± 5.8	25.4 ± 6.1	19.6 ± 4.8	0.2	0.7
Calcium (mg/dl)	8.5 ± 0.8	8.3 ± 0.7	8.3 ± 0.8	0.2	0.7
Phosphorus (mg/dl)	5.1 ± 0.7	5.2 ± 1.3	4.6 ± 0.7	0.7	0.4
iPTH (pg/dl)	397.6 ± 58.2	534.8 ± 132.4	380.5 ± 54.7	0.3	0.6
Homocysteine (nmol/ml)	8.5 ± 2.1	8.7 ± 2.1	8.4 ± 1.9	0.02	0.9
Paraoxonase 1 (ng/ml)	41.1 ± 10.1	45.6 ± 11.2	48.8 ± 11.8	0.3	0.6
Cholesterol (mg/dl)	175.5 ± 23.3	178.03 ± 24.3	178.6±41.06	0.01	0.9
TG (mg/dl)	126.4 ±31.2	154.1 ± 37.9	121.5 ± 29.8	1.5	0.2
HDL (mg/dl)	39.8 ±9.8	35.8 ± 4.1	43.5 ± 10.6	0.9	0.4
LDL (mg/dl)	115.4 ± 28.6	112.2 ± 27.9	103.5 ± 23.8	0.6	0.5
Creatinine (mg/dl)	4.7 ± 1.1	4.8 ± 1.2	4.6 ± 1.1	0.02	0.9
eGFR (ml/min/1.73m^2)	17.3 ±4.2	12.9 ± 3.1	13.9 ± 3.3	0.9	0.3
CIMT (mm)	1.1 ± 0.2	1.2 ± 0.3	1.1 ± 0.2	0.3	0.6

*Significant; **Highly significant

There was significant statistical highly positive correlation of paraoxonase 1 with hemoglobin and eGFR But, there was significant statistical highly negative correlation with CRP, PO_4, creatinine, homocysteine, LDL and CIMT & significant negative correlation with BMI, $Ca^{2+}$and iPTH, While, there was no correlation with age, cholesterol, TG and HDL (Table 6).

Table (6): Correlation between serum Paraoxonase 1 (PON 1 activity) and different parameters in all studied CKD patients (group (2) predialysis, and group (3) on HD) (n=65)

Variables	Paraoxonase 1 (ng/ml)	
	r	P
Age /years	0.03	0.7
BMI (kg/m^2)	-0.21	0.04*
Hb (g/dl)	0.3	< 0.0001**
Calcium (mg/dl)	-0.23	0.03*
Phosphorus (mg/dl)	-0.31	0.002**
iPTH (pg/dl)	-0.2	0.05*
Homocysteine (nmol/ml)	-0.39	< 0.0001**
Cholesterol (mg/dl)	-0.9	0.3
TG (mg/dl)	-0.0001	0.9
HDL (mg/dl)	-0.16	0.1
LDL (mg/dl)	-0.41	< 0.0001**
CRP (mg/dl)	-0.24	0.01**
Creatinine (mg/dl)	-0.39	< 0.0001**
eGFR (ml/min/1.73 m^2)	0.49	< 0.0001**
CIMT (mm)	-0.4	< 0.0001**

*Significant; **Highly significant

There was significant statistical highly positive correlation of CIMT with BMI, CRP, Ca^{2+}, PO_4, iPTH, homocysteine, creatinine and LDL. But, there was significant statistical highly negative correlation with Hb, eGFR and paraoxonase 1. While, there was no correlation with age, cholesterol, TG and HDL (Table 7).

Table (7): Correlation between CIMT and different parameters in all studied CKD patients (group (2) predialysis, and group (3) on HD) (n=65)

Variables	CIMT (mm)	
	r	P
Age /years	0.04	0.6
BMI (kg/m^2)	0.2	< 0.0001**
Hb (g/dl)	-0.51	< 0.0001**
Calcium (mg/dl)	0.55	< 0.0001**
Phosphorus (mg/dl)	0.36	< 0.0001**
iPTH (pg/dl)	0.24	0.01**
Homocysteine (nmol/ml)	0.56	< 0.0001**
Cholesterol (mg/dl)	-0.9	0.3
TG (mg/dl)	0.1	0.1
HDL (mg/dl)	-0.89	0.3
LDL (mg/dl)	0.41	< 0.0001**
CRP (mg/dl)	0.38	< 0.0001**
Creatinine (mg/dl)	0.6	< 0.0001**
eGFR (ml/min/1.73m^2)	-7.9	< 0.0001**
Paraoxonase 1 (ng/ml)	-0.4	< 0.0001**

*Significant; **Highly significant

The significant independent risk factor for atherosclerosis in all studied CKD patients (predialysis & on HD) was paraoxonase 1 activity (Serum PON 1) (Table 8).

Table (8): Binary (Univariate) Logistic Regression analysis of the predictors of atherosclerosis in all studied CKD patients (group (2) predialysis, and group (3) on HD) (n=65)

	Odds Ratio	Sig.	95% C.I. for Odds ratio
BMI (kg/m^2)	0.8	0.38	25 - 29.2
Hb (g/dl)	0.01	0.9	8.5 – 9.2
Calcium (mg/dl)	1.7	0.18	7.5 – 9.2
Phosphorus (mg/dl)	1.2	0.2	5.4 – 9.3
iPTH (pg/dl)	0.4	0.8	132 - 413
Homocysteine (nmol/ml)	1.5	0.2	8.9 – 22.1
Paraoxonase 1 (ng/ml)	3.9	0.05*	41.8 - 148
LDL (mg/dl)	0.13	0.7	92 - 170
eGFR (ml/min/1.73 m^2)	1.6	0.19	11 - 23

*Significant; **Highly significant.

The most common cause of CKD in the studied patients was hypertension (40%) followed by combined HTN & DM (16.9%) then DM (13.8%) & others (Figure 1).

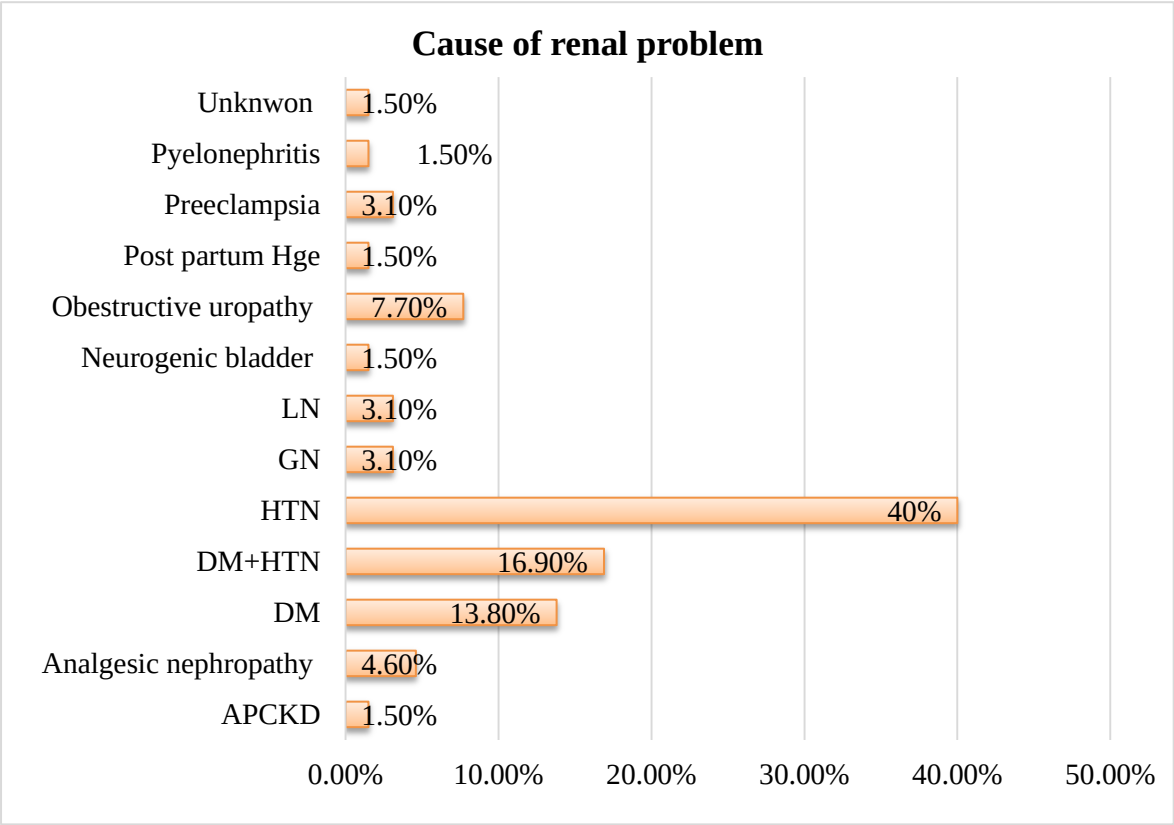

Figure (1): The cause of the renal disease of the studied patients [HTN, Hypertension; DM, Diabetes mellitus; GN, Glomerulonephritis; LN, lupus nephritis; APCKD, Adult polycystic kidney disease; Hge, hemorrhage.

Serum paraoxonase 1 at a cut off level ≤ 53.3 can detect the presence of atherosclerosis in CKD patients with 80% sensitivity and 73% specificity. AUC = 0.7 (P value = 0.0001) (Figure 2).

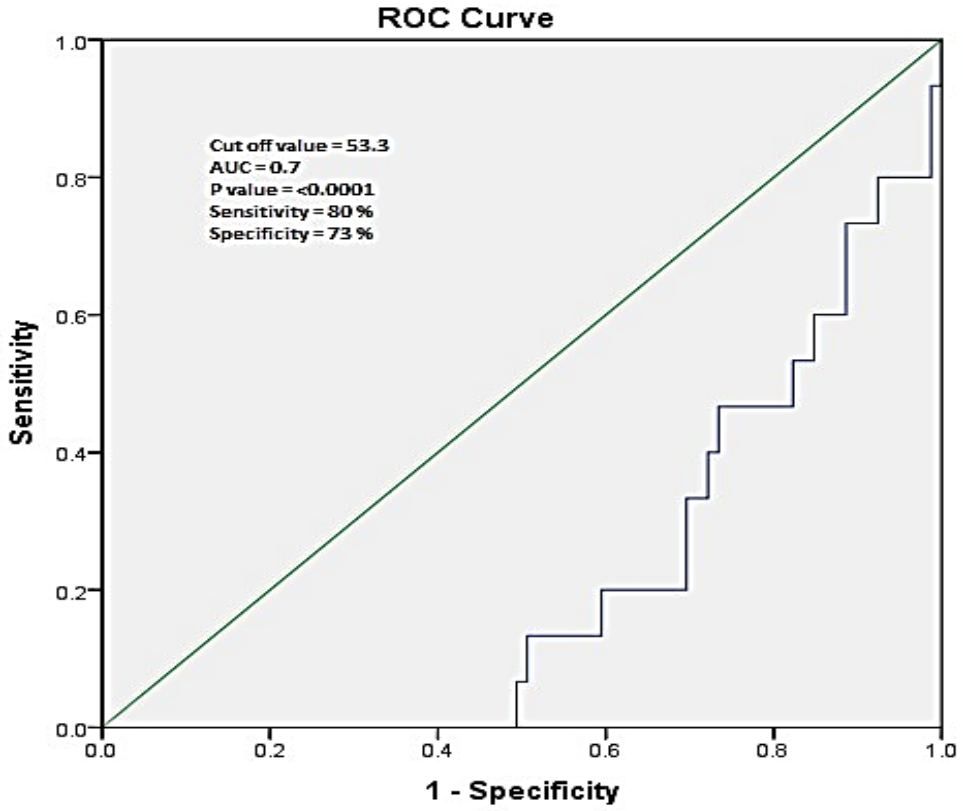

Figure (2): Cut off value of serum paraoxonase as marker of atherosclerosis in CKD patients.

DISCUSSION

As a prevalent ailment linked to a higher risk of CVD and CRF, CKD is acknowledged as a global public health concern. The primary cause of death and morbidity for CKD patients is CVD [(9)]. Compared to age-mathed controls, patients with CKD have a tenfold increased risk of getting CVD. Being in charge of 54% of ESRD10 patient fatalities [(10)]. Patients with all stages of CKD have an increased prevalence of CVD. The study of the Cardiovascular Health Study showed that the risk of CVD and all-cause mortality rose by 5% and 6%, respectively, with every 10 mL/min per 1.73 m^2 drop in GFR [(9)].

Early stages of CKD are influenced by traditional risk factors such as obesity, diabetes, dyslipidemia, and hypertension that are linked to CV disease. The main factors accelerating the progression of CV disease in these patients are non-traditional risk factors, such as accumulation of a class of endogenous compounds called "uremic toxins," inflammation, malnutrition, anemia, volume expansion, an osmotic and non-osmotic sodium retention, and a variety of hormonal disorders [(11)].

Hemodialysis (HD) patients with CKD are subject to many risk factors for atherosclerosis, including endothelial dysfunction, oxidative stress, and dyslipidemia. Low blood levels of HDL-C, higher triglyceride levels, and elevated triglyceride-rich lipoproteins or lipoprotein remnants are the major indicators of dyslipidemia [(8)].

Because of its lipid-modifying capabilities, antioxidant activity, anti-inflammatory, anti-apoptotic, anti-thrombosis, and anti-adhesion qualities, PON1 is known to prevent atherosclerosis. Patients with uremia who need HD are particularly vulnerable to atherosclerosis and its after effects [(12)].

In the present study, CKD patients had higher level of TG, LDL-C and a lower level of HDL-C compared to healthy subjects as in previous studies [(13-15)]. **Elarbagy *et al.*** [(10)] and **Nawaka *et al.*** [(16)], concluded that TGs and LDL-C were significantly higher in CKD patients than in non-CKD patients. Moreover, **Abd elhady *et al.*** [(9)] stated that there was significant increase in serum triglycerides in CKD patients (predialysis & on HD) compared to the control group.

In our study, patients with CKD had higher phosphorus and iPTH but lower calcium levels than healthy subjects. Similarly, other researchers revealed that high levels of PTH and hyperphosphatemia, accompanied by hypocalcemia, were found statistically significant in CKD patients compared to the control group [(17, 18)].

According to our research, CKD-HD patients had considerably higher CRP than controls when compared to CKD predialysis. **Saeed *et al.*** [(19)] observed a similar finding.

Our study revealed that the mean value of serum PON 1 (PON1 activity) among the CKD patients on HD (29.7 ± 9.5 ng/ml) was significantly lower than that of CKD predialysis patients (60.5 ± 35.3 ng/ml), which in turn was statistically lower than that of the healthy subjects group (78.3 ± 37.6 ng/ml); (p value=0.000). PON 1 level significantly decreased when GFR (CKD patients) was decreased, as previously shown by **Miljkovic *et al.*** [20] and **Prakash *et al.*** [13]. Similarly, others found that paraoxonase activity was lower in CKD patients than in healthy persons [21-23]. Furthermore, serum PON activity in CKD is decreased, which is especially evident in patients receiving hemodialysis, according to **Solati and Mahboobi** [24]**.** Also, PON1 was considerably lower in hemodialysis patients than in controls, according to further research [8, 25, 26].

In our investigation, we found that the CKD patients with HD had serum homocysteine (Hcy) levels (10.7 ± 4.2 nmol/ml) that were statistically greater than those of the CKD predialysis group (5.9 ± 2.9 nmol/ml), and even higher than those of the healthy participants (4.7 ± 2.3 nmol/ml) (p value=0.000). Therefore, compared to controls, CKD patients had considerably higher blood Hcy levels. This result is consistent with prior research by **Kruglova *et al.*** [27] who found that the CKD patient group had significantly higher blood homocysteine levels than the healthy control group.

Our study showed that, LVH and increased CIMT were present in CKD patients more than in healthy subjects. As regards CIMT, CIMT mean values among the CKD on hemodialysis patients (1.2 ± 0.3 mm) were statistically higher than that among the CKD predialysis group (1.02 ± 0.2 mm), which were statistically higher than those among the healthy subjects' group (0.5 ± 0.07 mm). This agrees with other investigators [9,10]. Also**,** previous studies showed that the mean CIMT was higher in CKD population compared to controls [14,15].

Regarding LVH, it was found in 43.33% of pre-dialysis CKD patients, and in 62.86% of patients receiving haemodialysis. **Zanib *et al.*** [28] showed that high prevalence of LVH in the dialysis population.

Our study showed that paraoxonase genotype QQ (AA) and allele Q (A) were common in CKD and ESRD than in healthy subjects group. Similarly, **Ribeiro *et al.*** [26] showed that QQ genotype was the most frequent genotype found in hemodialysis patients. **Nawaka *et al.*** [16] observed that there was no discernible difference in the genotype and allele frequencies of the PON1 Q192R polymorphisms between patients with and without CKD. The polymorphisms (192 Gln/Arg) were evaluated in 136 normal controls and 96 hemodialysis patients, according to **Itahara *et al.*** [29]. The distribution of gene polymorphism did not differ between the control group and the sick group.

There was no discernible correlation between the genotype distribution of the PON1 Q192R polymorphisms and (BMI or levels of Hb, CRP, Calcium, Phosphorus, iPTH, Homocysteine, Paraoxonase 1, Cholesterol, TGs, HDL, LDL, Cr & eGFR or CIMT). Similarly, **Itahara *et al.*** [29] showed that, regardless of genetic variation, PON1 enzyme activity declined in hemodialysis patients.

The relationship between paraoxonase and BMI was statistically significant and unfavorable. **Mohammed *et al.*** [22] also showed a correlation between an elevated body mass index and lower circulating PON lactonase activity in individuals with chronic kidney disease.

The amount of serum paraoxonase 1 showed a significantly substantial positive connection with Hb. Similarly, **Okuturlar *et al.*** [30] demonstrated that serum PON levels were considerably lower in anemic CKD patients.

The results showed a strong inverse relationship between hsCRP and paraoxonase. According to Ribeiro ***et al.*** [26]**, Lahrach *et al.*** [31]**,** found that, in hemodialysis patients, PON 1 activity and hsCRP showed a substantial negative connection,

Paraoxonase and LDL cholesterol showed a highly significant negative link, but there was no correlation with cholesterol. HDL-C, TGS. **Lahrach *et al.*** [31] found that in hemodialysis patients, PON 1 activity and LDL cholesterol had a substantial negative connection. PON1 level is positively connected with HDL cholesterol and adversely correlated with LDL cholesterol, according to **Kota *et al.*** [32]. Furthermore, PON1 concentration was found to be substantially and favorably linked with HDL-C by **Samouilidou *et al.*** [8]**.** In addition, PON1 was found to be adversely associated with LDL-C in hemodialysis patients. A highly statistical negative correlation between paraoxonase and homocysteine was documented. This result agrees with **Locsey *et al.*** [25]**.** As previously discovered by **Miljkovic *et al.*** [20]**,** there was a highly substantial negative connection between paraoxonase 1 activity and creatinine levels. A strong statistically significant positive connection was observed between paraoxonase and eGFR. Likewise, **Watanabe *et al.*** [21] confirmed that the paraoxonase activity in CKF, an advanced stage of CKD, was lower than in non-CKF.

A highly significant positive correlation of CIMT with BMI was found as previously described by **Abbasi *et al.*** [33] In contrast, **Okasha *et al.*** [34] stated that CIMT was in a negative correlation with body mass index. There was highly significant positive correlation of serum CIMT with LDL & no significant association with total cholesterol, TGs and HDL-c. **Abbasi *et al.*** [33] approved that low-density lipoprotein in HD patients exhibited a strong correlation with their CIMT. A highly significant positive correlation of serum CIMT with homocysteine was presented. This result agrees with **Lai *et al.*** [35] who showed an increase in homocysteine with a positive correlation with the increase of cIMT in CKD patients. There was

highly significant positive association of serum CIMT with calcium, phosphorus & significant correlation with iPTH. This agrees with that of **Okasha *et al.* [34]** who showed that cIMT was in a strong correlation with PTH and phosphorus. Serum CIMT and hsCRP showed a significantly significant positive connection. This outcome is somewhat similar to **Mohamed *et al.* [21]**. A significant negative correlation of CIMT with paraoxonase 1 level was found as previously described by **Okuturlar *et al.* [30]. Saeed *et al.* [19]** showed that PON1 activity is a significant factor in determining IMT in individuals with CRF and that a low level of PON1 activity is linked to a higher risk of carotid IMT progression.

Our study showed that paraoxonase level (PON1 activity) is an independent risk factor of atherosclerosis in CKD patients. **Efe *et al.* [23]** proved that there was a substantial correlation between reduced aortic functions and blood paraoxonase-1 levels. The study's findings demonstrated the relationship between atherosclerosis and unfavorable cardiovascular events and serum paraoxonase-1 activity.

CONCLUSION

Serum paraoxonase 1 level (PON1 activity) was lower in CKD patients (predialysis & on HD). LVH and increased CIMT was more evident in CKD patients. Paraoxonase Q192R Genotype QQ and allele Q were common in CKD and ESRD and can be related to cardiovascular mortality in patients with CKD. Paraoxonase 1 was an independent risk factor for atherosclerosis in CKD. Thus, it may serve as a potential biomarker in CRF in relation to CVD. Associations of PON1 with dyslipidaemia, LVH & higher CIMT are equip evidences for accounting PON1 as a therapeutic purpose in the inhibition of atherosclerosis and its side effects in patients with uremia.

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