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RESEARCH ARTICLE

THE IMPACT OF RS662 GENOTYPING ON THE SERUM ACTIVITY OF THE PARAOXONASE -1 ENZYME IN IRAQI PATIENTS WITH CARDIOVASCULAR DISEASES.

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Abstract

Background: Cardiovascular diseases (CVDs) are diseases that affect the heart or vessels. Coronary artery disease (CAD) and peripheral (PAD) are types of CVD. PON1 is a HDL-associated lipolactonase is considered to be atheroprotective by preventing LDL and cell membranes oxidation. A common polymorphism due to an amino acid substitution (Glutamine → Arginine) at codon 192 is considered to be a major determinant of variation in serum PON1 activity. Recent studies have suggested that the PON1192 polymorphism is an independent risk factor for CVD

Objectives: The effect of the PON1 activity and PON1 Q192R (rs662) polymorphisms on the CVD include patients with PAD and CAD.

Subject and Methods: This case control study consisted of 180 subjects were divided into 100 patients with CVD (50 patients with PAD and 50 patients with CAD), and 80 healthy controls. Serum levels for lipid profile and PON1 enzyme were measured by spectrophotometer using paraoxon as a substrate. The genetic polymorphism of PON1 was detected by TaqMan-based allele discrimination RT-PCR

Results: The means \pm SEM of serum PON1 activity were highly significantly decreased in PAD and CAD as compared to control group. The heterozygote (Q192R) genotype of PON1 polymorphism is abundant among all of the studied groups (68% for PAD, 56% for CAD and 65% for control, respectively). The CAD patients carry the high risk RR genotyping (odd ratio of 2.69, with the confidence interval of (1.12-6.47; $p=0.02$). The PAD who carry the RR genotype have a risk ratio 1.57 at confidence interval of 0.61- 4.02.

Conclusion: Reduction of PON1 activity is one of the risk factor of development of CVD. Patients who carry the RR genotyping were at high risk to develop CVD. Our results indicate that controversial relationships regarding the PON1 rs 662 (Q192R) polymorphism and CVD. Serum PON1 activity is more informative than the PON1 genotype in evaluating the severity and extent of cardiovascular disease in Iraqi patients.

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Introduction:-

Cardiovascular diseases (CVDs) are diseases that affect the heart or blood vessels, which include Coronary artery disease (CAD), Stroke, Peripheral arterial disease (PAD) and aortic disease (WHO,2015). Atherosclerosis is the primary cause of the development of cardiovascular diseases that account for the majority of fatal illnesses in developed countries and illness worldwide (Roth *et al.*, 2015). Genetic is fixed factor to CVD (WHO, 2012).

The high density lipoprotein (HDL) has a central role in the inhibition of atherosclerosis development due to its anti-atherogenic properties. The HDL is crucial for the reverse cholesterol transport (Cohen *et al.*, 2016), the process by which cholesterol is transferred from peripheral tissues, including the arterial wall, to the liver for excretion in the bile. The beneficial functions of HDL could be played by enzymes and proteins associated with this lipoprotein, such as cholesteryl ester transfer protein (CETP) and PON1 enzyme. In addition to playing a pivotal role in reverse cholesterol transport, The cardio protective role of the small dense HDL is done to its ability to accept cholesterol from foam cells by ATP binding cassette transporter A1 (ABCA1) (Du ,2015) . The HDL actually metabolizes lipid hydroperoxides preventing their accumulation, consequently impeding the atherogenic structural modification of LDL (Mackness *et al.*, 1991).This effect is related to enzymatic activity associated with HDL, and not chain-breaking antioxidants or transition metal chelation (Mackness *et al.*, 1993; Durrington *et al.*, 2001).

Human serum paraoxonase-1 (PON1: EC 3.1.8.1) is a 354 amino acid glycoprotein with a molecular weight of 43 KD (Adkins *et al.*,1991). PON1 is synthesized in the liver and secreted into the blood, where it associates exclusively with high-density lipoprotein (HDL) (Mackness *et al.*,1996) . PON1 is named for its ability to hydrolyze organophosphates such as paraoxon (Aldridge,1953) . The gene for PON1 is located between q21.3 and q22.1 on the long arm of chromosome 7 in humans (Harel *et al.*, 2004). PON1 is the first discovered member of the paraoxonase (PON) multi-gene family which comprises 3 members, PON1, PON2 and PON3, the genes for which are located adjacent to each other (Primo-Parma *et al.*, 1996).

The PON1 have significant anti-oxidative and anti-inflammatory properties through its enzymatic lactonase, peroxidase and esterase activities (Précourt *et al.*, 2011). PON1 is a HDL-associated lipolactonase is considered to be atheroprotective by preventing LDL and cell membranes oxidation (Altuner, 2011; Mackness, 2014). PON1 a major antioxidant, is regarded as a cardiovascular protective factor and its activity is reduced in patients with CVD (Deakin, *et al.*, 2004 and Kowalska *et al.*, 2015).

PON1 may slow the evolution of the early stages of atherosclerosis, and may participate in the reduction of cardiovascular risk through its activities, depending on genetic and environmental factors (Rainwater *et al.*, 2009).

The SNP rs662 present in the coding region, and causes exchange of amino acid from glutamine (Q) to arginine (R) at codon 192 (Q192R) ,the exchange of codon CAA to CGA in exon six of PON1gene determines the isoforms of the PON1 enzyme which differ greatly in the rate of hydrolysis of some substrates. Paraoxon is hydrolyzed at faster rate by the 192 R isoform compared to the Q192, but some organophosphates and lactones are hydrolyzed at greater rate by Q192 (Billecke *et al.*,2000; Davies *et al.*, 1996) . The R isoform of the enzyme has higher lactonase activity and increased antiatherogenic potential (Gaidukov *et al.*, 2006).The polymorphism Q192R (rs662) leads to different PON1enzymatic activity behavior dependent of the isoenzyme that is present (Costa *et al.*,2003).

Material and Methods:-**Subjects:-**

This research was a case-control study .It was conducted in Coronary Care Unit (CCU) of Gazi Al-Harery Hospital for Specialized Surgery in Iraqi Center for Heart Diseases, Ibn Al-Betar Hospital of Cardiovascular Diseases, and Al-Immamain Al-Kadhmain Medical City. The genetic practical part of this study was conducted at Department of Chemistry and Biochemistry Laboratory, and study protocol was approved by Ethical Committee of College of Medicine, Al-Nahrain University, Baghdad, Iraq. In this study 180 subjects were examined during the period from February 2016 to October 2016. Out of these 180 subject 100 patients were with CVD (50 patients with CAD and 50 patients with PAD) , the other 80 subjects were healthy controls . The ages of all subjects were ranging from 35 to 65 years.

Coronary artery disease group (CAD):-

Included 50 patients with unstable angina (UA) based on the defined electrocardiographic changes and non elevated cardiac enzymes such as CK-MB and Troponin, or angiographic evidence of at least 50% stenosis of one or more epicardial vessels.

Peripheral artery disease group (PAD):-

Included 50 patients were caused by atherosclerosis that leads to stenosis (narrowing) or blockage in the major vessels supplying the lower extremities. The ankle to brachial pressure index (ABPI) is measured using a sphygmomanometer.

All patients were diagnosed by Senior Cardiologist and underwent clinical examination, ECG, Eco-cardiograph and angiographic study.

Control groups:-

Include 80 healthy individuals with no history of diabetes, hypertensive, hyperlipidemia or coronary vascular disease.

Patients Exclusion criteria:-

Patients with renal failure or nephropathy, with hepatic diseases, and patients on anti-depressant and anti-psychotic drug

Blood sample Collection:-

About six milliliters of venous blood was taken from each participant, then was divided as following: 4 ml of blood were put into serum separating tube (SST) and left to clot for 30 min at room temperature then were centrifuged at 4000 rpm for 10 min. The serum separated was distributed in small aliquots and stored at -80°C for the evaluation of PON1 enzyme activity and analysis of total cholesterol(TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein (LDL-C), triglyceride(TG), and 2 ml of the blood was put into two separated EDTA containing tubes for the assay of DNA extraction and genotyping for the detection of the Q192 R polymorphism of PON1 (rs662) gene using RT-PCR technique.

Determination of serum PON1 enzyme Activity:-

Serum PON1 activity was measured using a procedure modified from the method of Krisch (1968). It depends on monitoring the liberation of p-nitrophenol upon PON1 enzymatic hydrolysis of paraoxon (Sigma-Aldrich, USA) and the absorbance was continuously monitored for 6 min at 405 nm against paraoxonase substrate reagent solution which was used as a reagent blank for the correction of not enzymatic hydrolysis of paraoxon. The reaction is stable up to 5 minute. A molar extinction coefficient of 18.05×10^3 was used for the calculations of PON1 activity (Biggs, 1954), and units of enzyme activity were expressed as micromoles of paraoxon hydrolyzed per minute. The paraoxonase assay contained 760µl of assay buffer (0.132M Tris-HCl, pH=8.5, 1.32 mM CaCl₂, and 2.63 M NaCl), 40µl of fresh serum, and 200µl of 6 mM freshly prepared paraoxon substrate solution. A stock substrate solution of 120 mM paraoxon in acetone was stable for approximately 3 wk. The stock solution was diluted 1:20 with 50mM Tris-HCl (pH=8.5) for each set of assays. The assay at 37°C was initiated by the addition of the substrate solution, and the absorbance was continuously monitored at 405 nm.

Determination of serum lipids profile:-

Total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) were determined by using CHOD-PAP method using kit from (Randox, UK), triglycerides (TG) was measured by the enzymatic GPO-PAP method using kit from (Randox, UK). LDL was estimated using Friedwald formula: $LDL = TC - (HDL + TG/2.2)$ mmol/l (Friedewald *et al.*, 1972).

Genotyping of PON1 Q192R:-

Genomic DNA was isolated from whole peripheral blood using DNA extraction kit (Geneaid, Taiwan) according to the manufacturer's protocol. Q192R polymorphisms of the PON1 (rs662) gene were determined by using TaqMan-based allele discrimination real time PCR based on the following

Primers :5- ACCTGAGCACTTTTATGG -3 forward primer and 5- ATCAA TCCT TCTG CCAC -3 reverse primer were designed by (Alpha DNA, USA); and

Probes: GoTaq® Probe qPCR Master Mix :- PON1 probe-1: Cy5-CCCCTACTTAC~~A~~ATCCTGGGAGA-BHQ2 (wild), and PON1 probe -2: Cy3-CCCCTACTTAC~~G~~ATCCTGGGAGA-BHQ2 (mutant). were designed by (promega, USA). Genotyping assays were performed on Aligent RT- PCR (Stratagene 3005xp - USA).

The SNP rs 662 at exon 6 of the PON1 gene causes change in the base sequenced (missense) at the codon number 672 from CAA to CGA at mRNA. This base shift result in replacement of the amino acid in the sequence at position 192 (Glutamine to Arginine) of the resulting polypeptide chain of the PON1 enzyme (Q192R).

Statistical Analyses:-

The data were analyzed using SPSS (version 23.0) software for windows (USA). Comparisons between groups were performed using one-way ANOVA for multiple comparisons. Chi- square test was performed for Q192R polymorphism in PON1(rs662) gene to determine if the studied samples demonstrated Hardy Weinberg equilibrium (H-WE) . Logistic regression was used to calculate odd ratio (ORs) at confidence interval (CI) of 95%. All results were interpreted at an alpha level of 0.05.

Results:-

Results of general characteristics and study biochemical and clinical parameters:-

The clinical characteristics of the study groups are shown in table (1)

The mean \pm SEM of Age (year) for patients groups (PAD and CAD) and control group were 59.60 ± 0.63 year, 53.94 ± 0.92 year, and 54.71 ± 0.92 years respectively .The mean age of the PAD patients group were significantly higher ($P < 0.001$) than control and CAD groups whereas the mean age of CAD patient group were no significantly different ($P = 0.55$) when compared to those of PAD group. The mean \pm SEM of BMI (Kg/m^2), systolic blood pressure (mmHg), and diastolic blood pressure (mmHg) for PAD patients ($29.37 \pm 0.30 \text{ kg}/\text{m}^2$, $143.50 \pm 0.93 \text{ mmHg}$, $92.70 \pm 1.99 \text{ mmHg}$, respectively) , CAD patients ($30.32 \pm 0.43 \text{ kg}/\text{m}^2$, $143.22 \pm 0.94 \text{ mmHg}$, $92.68 \pm 0.77 \text{ mmHg}$, respectively) and control group were ($25.59 \pm 0.22 \text{ kg}/\text{m}^2$, $125.79 \pm 0.82 \text{ mmHg}$, $82.25 \pm 0.38 \text{ mmHg}$, respectively).

Both PAD and CAD patient groups have high significant increases ($P < 0.001$) in mean of BMI (Kg/m^2), systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) when compared with control group. Yet, there was no significant increase in the mean BMI ($P = 0.07$) in CAD as compared to that of the PAD group.

Table 1:- Descriptive statistics of biochemical & clinical parameters in the study groups

Parameters	PAD (n=50)	CAD (n=50)	Control (n=80)
Age (year)	$59.60 \pm \triangle^{a***} \triangle^{b***}$ 0.63	$53.94 \pm$ 0.92	$54.71 \pm$ 0.92
BMI (kg/m^2)	$29.37 \pm \triangle^{a***}$ 0.30	$30.32 \pm \triangle^{a***}$ 0.43	$25.59 \pm$ 0.22
SBP (mmHg)	$143.50 \pm \triangle^{a***}$ 0.93	$143.22 \pm \triangle^{a***}$ 0.94	$125.79 \pm$ 0.82
DBP (mmHg)	$92.70 \pm \triangle^{a***}$ 1.99	$92.68 \pm \triangle^{a***}$ 0.77	$82.25 \pm$ 0.38
TC (mmol/l)	$4.49 \pm \triangle$ 0.10	$4.43 \pm \nabla$ 0.09	$4.44 \pm$ 0.04
HDL-C (mmol/l)	$0.81 \pm \nabla^{a***} \nabla^{b*}$ 0.03	$0.93 \pm \nabla^{a***}$ 0.35	$1.28 \pm$ 0.02
TG (mmol/l)	$1.43 \pm \triangle^{a***}$ 0.06	$1.36 \pm \triangle^{a***}$ 0.04	$1.12 \pm$ 0.03
VLDL-C (mmol/l)	$0.65 \pm \triangle^{a***}$ 0.03	$0.62 \pm \triangle^{a***}$ 0.02	$0.51 \pm$ 0.01
LDL-C (mmol/l)	$3.04 \pm \triangle^{a***}$ 0.09	$2.88 \pm \triangle^{a*}$ 0.09	$2.66 \pm$ 0.05
TC/HDL-C	$6.17 \pm \triangle^{a***}$ 0.36	$5.28 \pm \triangle^{a***}$ 0.34	$3.59 \pm$ 0.08

PON1 activity (U/ml)	42.69± ^{▼ a*** ▼ b*} 2.06	48.96± ^{▼ a***} 1.95	85.48 ± 2.16
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PAD: Peripheral artery disease group; CAD : Coronary artery disease group; Control group; SEM: standard error of the mean
a= ANOVA test between PAD or CAD patients group versus control group: ^{▼ or ▲}=significant decrease or increase ***: (P<0.001); **: (P<0.01);
 *: P<0.05. **b**= ANOVA test between PAD versus CAD patients groups: ^{▼ or ▲}=significant decrease or increase ***: (P<0.001); **: (P<0.01);
 *: P<0.05.

The mean ± SEM of serum lipid profile (TC, HDL-C, VLDL-C, LDL-C) and atherogenic index (TC/HDL-C ratio), are listed in table (1). Statistical analysis revealed no significant difference in the mean values of TC between patient groups and control group ($P > 0.05$) and there were highly significant decrease in the mean of HDL in PAD (0.81 ± 0.03 mmol/l) and CAD (0.93 ± 0.035 mmol/l) as compared to the control group mean values (1.28 ± 0.02 mmol/l, $P < 0.001$).

The means of serum TG and VLDL-C concentration were highly significant increased ($P < 0.001$) in PAD (1.43 ± 0.06 mmol/l, 0.65 ± 0.03 mmol/l) and in CAD (1.36 ± 0.04 mmol/l, 0.62 ± 0.02 mmol/l) as compared to those of the control group (1.12 ± 0.03 mmol/l, 0.51 ± 0.01 mmol/l). The TC/HDL-C concentration were highly significantly increased in both patient groups but LDL-C is significantly elevated only in PAD group (3.04 ± 0.094 mmol/l, $P < 0.001$) but is near significantly increased in CAD patients (2.88 ± 0.098 mmol/l, $P = 0.057$) as compared to control group.

The means of serum PON1 activity were highly significantly decreased ($P < 0.001$) in PAD (42.69 ± 2.06 U/ml) and CAD (48.96 ± 1.95 U/ml) as compared to mean of PON1 of the control group (85.48 ± 2.16 U/ml). Moreover, the PON1 activity were decreased in PAD group as compared to those of the CAD group ($P = 0.03$).

Results of Molecular Analyses:-

The Hardy-Weinberg distributions of PON1 genotypes Q192R among all of the patient and control groups are represented in table (2) and figure (1). Using X^2 test, the highest frequency of PON1 genotype Q192R in patients and control groups were (68% for PAD, 56% for CAD, and 56% for control respectively). Hardy Weinberg equilibrium (H-WE) delineated that highest allele frequency is 192R in both PAD and CAD groups (0.54, 0.58, respectively), Whereas, the 192Q allele is of highest percentage in the control group as compared to the same group distribution frequency of 192R allele (0.54).

Table 2:-Distribution of genotypes and alleles frequency of the PON1 rs662 (Q192R) polymorphism among different studied groups

Groups	Genotypes frequency			Alleles frequency	
	Q192Q, n(%)	Q192R, n(%)	R192R n(%)	192Q	192R
PAD (n=50)	6(12%)	34(68%)	10(20%)	0.46	0.54
CAD(n=50)	7(14%)	28(56%)	15(30%)	0.42	0.58
Control(n=80)	17(21.3%)	52(65%)	11(13.7%)	0.54	0.46

Chi square test : $P = 0.161$

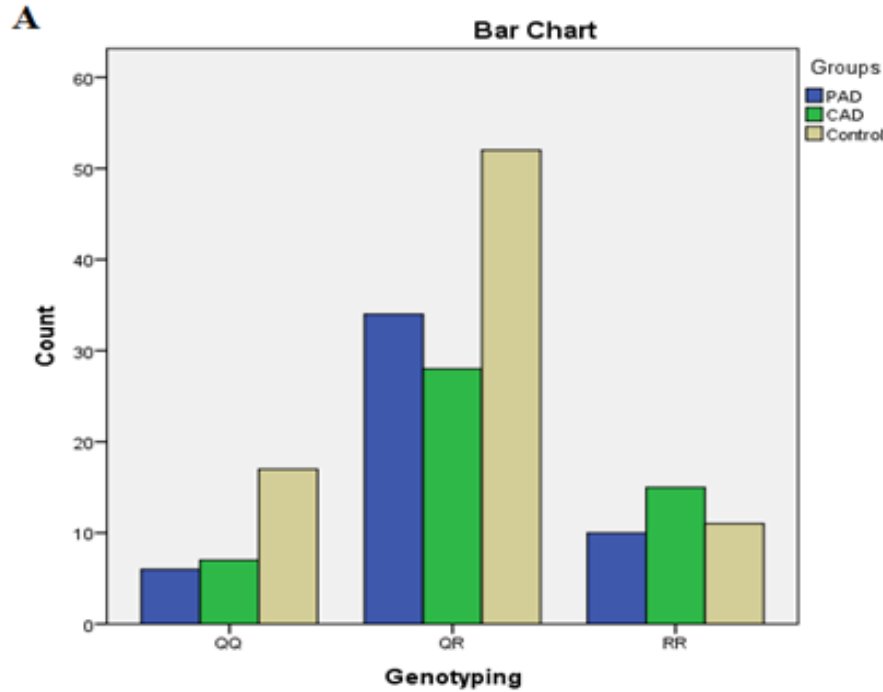


Figure 1:- The distribution of studied samples among different groups according to Genotypes

Table 3:- The Odds ratio, 95% confidence interval of the PON1 rs662 (Q192 R) polymorphism in PAD and CAD groups

Genotypes Q192R	PAD Odds Ratio(95%CI)	P-Value	CAD Odds Ratio(95%CI)	P-Value
QQ	Reference		Reference	
QR	1.14(0.54-2.43)	0.72	0.69(0.33-1.41)	0.30
RR	1.57(0.61-4.02)	0.34	2.69(1.12-6.47)	0.02
QR+RR	0.51(0.19-1.39)	0.18	0.60(0.23-1.58)	0.30

The odd ratio, 95% confidence intervals between PAD and CAD groups who carry the RR genotyping were at high risk (odd ratio of 2.69) with the confidence interval of 1.12-6.47(p=0.02) to develop CAD as compared to PAD individual, who carry other genotypes. The mean odd ratio 1.57 was found in PAD who carry the RR genotype at confidence interval of 0.61- 4.02. The combination of the abnormal genotypes (QR+RR) displayed higher frequency in patients with PAD 88% (44/50) and 86% (43/50) in CAD as compared to 78% (63/80) in the control group. Yet, the combined genotypes showed no significant change in the odd ratio in both patient groups ($P > 0.05$).

Table 4:- The distribution of PON1 enzyme activity among studied group according to genotyping of the PON1 rs662 (Q192R) polymorphism

Genotypes Q192R	PON1enzyme activity(U/ml)		
	PDA (n=50)	CAD (n=50)	Control (n=80)
QQ(n=30)	(n=6) 38.43±6.42 ^{a▼*** b*}	(n=7) 43.49±4.24 ^{a▼***}	(n=17) 90.86±4.57
QR(n=114)	(n=34) 42.39±2.49 ^{a▼*** b*}	(n=28) 49.71±2.79 ^{a▼***}	(n=52) 84.67±2.82
RR(n=36)	(n=10) 46.31±4.68 ^{a***}	(n=15) 50.11±3.39 ^{a▼***}	(n=11) 80.95±4.09

PAD: Peripheral artery disease group; CAD: Coronary artery disease group; Control group a= ANOVA test between PAD or CAD patients group versus control group: *** = ($P < 0.001$) ; ** : ($P < 0.01$); * : $P < 0.05$. b= ANOVA test between PAD versus CAD patients groups: *** : ($P < 0.001$) ; ** : ($P < 0.01$); * : $P < 0.05$.

Table (4) shows the distribution of serum PON1 enzyme activities among PAD, CAD, and control groups according to genotyping of the PON1rs662 (Q192R) polymorphism. The serum PON1 enzyme activity were significantly reduced ($P < 0.001$) in PAD and CAD patients who carry QQ (wild) genotype as compared with QQ genotyping of the control group.

Patients with PAD and CAD group who were heterozygote for PON1 activity (Q192R) were having significantly decreased PON1 activity ($P < 0.001$) as compared to same genotype in the control group. Highest PON1 enzyme activity was reported in the control group with RR genotype as compared to PAD and CAD patients carrying the same genotype ($P < 0.001$). Furthermore, the means PON1 activities were decreased in PAD patients who carries QQ ($P = 0.02$), QR ($P = 0.04$), and RR ($P = 0.07$) genotypes as compared to same genotype carrier in the CAD group.

Discussion:-

The means of age, BMI, systolic and diastolic blood pressure were significantly high in both PAD and CAD patient groups when compared to control group. These results are different from those of Paapstel *et al.* (2016) who reported no difference in age and mean diastolic blood pressure in PAD group but the CAD patients had significantly higher BMI levels than the PAD and the control group.

This study displayed a disturbance in the lipid profile as represented by highly significant decrease in the means of HDL-C, increase in TG, VLDL-C and TC/HDL-C in PAD and CAD. The mean LDL-C mmol/l concentration was highly significant increase in PAD patient group ($P < 0.001$) but it is significantly increased in CAD patient group. Ivanova *et al.* (2015) reported that individuals with high CVD risk have elevated amounts of circulating low-density lipoprotein (LDL) and decreased percentages of high-density lipoprotein (HDL).

The major source of cholesterol and the lipids is LDL that contributes to the development of the atherosclerotic plaque, whereas no atherogenic HDL and its concentrations were inversely correlated with the risk of CVD development. On the contrary, Paapstel *et al.* (2016) revealed no significant changes in HDL-C, TC, LDL-C in patients with PAD.

High TC/HDL-C is considered predictor to CVD, As TC/HDL-C ratio is considered a more sensitive and specific index of cardiovascular risk than total cholesterol, the Canadian working group has chosen this lipid ratio as a secondary goal of therapy (Genest *et al.*, 2003).

High-density lipoprotein cholesterol is considered the best biochemical predictor of PAD. Other risk factors of premature PAD include smoking, diabetes, and hypertension, high circulating levels of cholesterol, TG, and homocystein. Whereas, high blood fibrinogen concentration is a thrombosis risk factor (Dell'Omo *et al.*, 2014)

Determination of serum PON1 activities in PAD and CAD groups revealed highly significant loss of PON1 activities in these patients. Some authors have evidenced that PON1 enzyme activity is lower in subjects with significant coronary artery disease, and that there is a significant relationship between PON1 activity and coronary atherosclerosis determined by coronary angiography (Granér *et al.*, 2006). The PON1 activity in different CAD groups was significantly lower than those of non-CAD counter group and the decreases of PON1 activity were especially evident in severe CAD group compared with those with mild or moderate CAD. This finding reflects a close relationship with the risk of CAD (Lakshmy *et al.*, 2010).

PONs enzymes possess numerous atheroprotective properties, which include anti-oxidant activity, anti-inflammatory action, anti-apoptosis, anti-thrombosis, and anti-adhesion, also it maintains HDL function, and promotes cholesterol efflux (Chistiakov *et al.*, 2017).

These disorders are likely to contribute to increased plaque burden, plaque instability, and greater complexity of vascular disease (Thiruvoipati *et al.*, 2015). Moreover, the increase in the atherogenic index (TC/HDL-C) is reported to be the best biochemical predictor of PAD, according to the Trans-Atlantic Inter-Society Consensus Document on Management of Peripheral Arterial Disease (TASMCII) (Norgren *et al.*, 2007). Surprisingly, they recorded that the prevalence of PAD in rural black population of Uganda was higher in normotensives and in those with a normal

BMI (Kumar *et al.*, 2007). This study revealed a strong association of PON1 activity with LDL-C in the studied groups.

Genetic variations have a major impact on PON1 catalytic activity and amino acid 192 is an important active-site of the enzyme also, was anchoring on HDL surface (Lawlor *et al.*, 2004).

The highest PON1 genotype frequency observed in the studied patients (PAD and CAD) and control groups is the heterozygote (Q192R) genotype (68%, 56% and 65%, respectively). This genotype is in the Hardy-Weinberg distribution.

Patients who carry the RR genotyping were at high risk (odd ratio of 2.69) with the confidence interval of 1.12-6.47 ($p=0.02$) to develop CAD as compared to PAD individual, who carry other genotypes. The mean odd ratio 1.57 was found in PAD who carry the RR genotype at confidence interval of 0.61- 4.02. The combination of the abnormal genotypes (QR+RR) displayed higher frequency in patients with PAD 88%. On the contrary, Lebedy *et al.* (2014) reported that the frequency of QQ is the highest in the control group and the patients had a higher frequency of AB(QR) and BB (RR) genotype as compared to controls

The mean serum PON1 activity were significantly reduced ($P<0.001$) in PAD and CAD patients who carry QQ (wild) genotype as compared with QQ genotyping of the control group. Serum paraoxonase activity is higher in RR genotype carrier and is the lowest in QQ genotype. Paraoxonase QR variants showed intermediate activities between respective homozygous. Other scientist reported an association of the PON1192RR genotype with low HDL-C levels and a higher level of LDL-cholesterol (Rozek *et al.*, 2005; Blatter *et al.*, 2006).

The PON1Q192R genotype has an impact on the hydrolytic activity of PON1 toward the substrate paraoxone (Scherrer *et al.*, 2015). The QQ genotype, with low PON1 activity, may be adequate to prevent lipid peroxidation in young or elderly subjects without atherosclerosis. However, the low PON1-activity QQ genotype may have its antioxidant capacity surpass with advanced age, which probably compromises the PON1 function in concomitant with CHD (Sentí *et al.*, 2001).

The highest allele frequency is R in patients within the PAD and CAD (0.54, 0.58, respectively) groups whereas, the Q allele is of highest percentage in the control group. Yet, higher frequencies of Q allozymes (0.78) was previously reported by Zargari *et al.* (2016)

Hernández-Díaz *et al.* (2016) who revealed that the 192Q allele carriers are at high risk to develop CAD. On the other hand, in another study, Bounafaa *et al.* (2015) reported that the PON1 R allele is abundant in CHD patients, indicating that the PON1192 polymorphism may be a risk factor for atherosclerosis.

The increased risk of CVD in RR genotype carrier could be attributed to an increased susceptibility of LDL to oxidation, and an alteration in function and reduced the antioxidant effect of HDL (Dell'Omo *et al.*, 2014), the common PON1 192R allele may be a genetic risk factor for atherogenesis by inducing chronic low-grade inflammation (Luersen *et al.*, 2011). Low serum PON1 activity alleles are associated with a decreased transfer of lipids between lipoprotein particles (Ombres *et al.* 1998). Conversely, several other studies inconsistent results regarding the link between PON1 Q192R polymorphism and CVD (Kallel *et al.*, 2010). The RR genotype was more frequent in patients with CVD and was independently associated with the disease risk. In this context, the Iraqi population presents controversial relationships regarding the Q192R SNP and cardiovascular disease.

The 192 Q PON1 allozyme was shown to bind to the HDL particle with 3-fold lower affinity than the 192 R allozyme, consequently showing lower stability, and lipolactonase activity, and a modulatory effect on macrophage cholesterol efflux. Both evaluated paraoxonase activities were found the greatest in the group of patients with 192 Q allozyme and this fact should be helpful to explain the high level of LDL cholesterol in patients with 192 R allozyme (Kasprzak *et al.*, 2014).

In this study the means of serum PON1 paraoxonase activity was recorded marked decrease in all CVD patients and it is more evident in the PAD group independent of the PON1 192 genotyping (QQ, QR, and RR). Similar no influence of PON1 genotypes was recently reported by Martínez -Quintana *et al.* (2017) in patients with acute coronary syndrome (ACS). In this context, the Iraqi population presents controversial relationships regarding the

PON1 rs 662 (Q192R) polymorphism and CVD that might be referred to small sample size, ethnic alteration, recruiting criteria, interaction between gene to gene and gene to environments (Rodríguez-Esparragón *et al.*,2005;Rainwater *et al.*,2009; Luu *et al.*,2011;Précourt *et al.*,2011). Further studies on larger sample size and including ethnicity are recommended on CVD patients. It has been concluded that the determination of serum PON1 activity is more informative than the PON1 genotype in evaluating the severity and extent of cardiovascular disease.

Conclusion:-

Reduction of PON1 activity is one of the risk factor of development of CVD . Patients who carry the RR genotyping were at high risk to develop CVD. Our results indicate that controversial relationships regarding the PON1 rs 662 (Q192R) polymorphism and CVD. Serum PON1 activity is more informative than the PON1 genotype in evaluating the severity and extent of cardiovascular disease in Iraqi patients.

List of abbreviation:-

Abbreviations and Acronyms	
ABCA1_ATP binding cassette transporter A1	LDL-C _ low-density lipoprotein cholesterol
ABPI_ brachial pressure index	PON1 _ paraoxonase-1
BMI _ body mass index	PAD_ peripheral artery disease
CAD _ coronary artery disease	Q_ glutamine amino acid
CETP_ cholesteryl ester transfer protein	R_ arginine amino acid
CHD_ coronary heart disease	SBP _ systolic blood pressure
CK-MB_ Creatine kinase -MB	SEM_ standard error of the mean
CVD_ cardiovascular disease	TG_ triglycerides
DBP _ dystolic blood pressure	VLDL _ very-low-density lipoprotein
HDL-C _ high-density lipoprotein cholesterol	WHO_ World Health Organization

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