PARAOXONASE 1 Gene Polymorphism Relationship with Type 2 Diabetes Mellitus

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Abstract: **Background:** Human serum paraoxonase1 (PON1) an antioxidant enzyme closely associated with high density lipoprotein (HDL) has been implicated in the prevention of low density lipoprotein (LDL) oxidation and these may provide HDL-associated protection against atherosclerosis. The aim of this study is to investigate PON1 activity and its gene polymorphism in type 2 diabetes mellitus and its potential significance in the occurrence of diabetic complications. **Patients and Methods:** This study includes 60 subjects divided into: Twenty healthy subjects as a control (group I), Twenty diabetic patients without vascular complications (group II), and Twenty diabetic patients with vascular complications (group III). Laboratory investigations included: estimation of PON1 enzymatic activity by hydrolysis of paraoxon and PON1gene polymorphism by polymerase chain reaction (PCR) followed by polymorphism specific restriction enzyme digestion , other investigations included fasting and post prandial plasma glucose (FPG and PPPG), glycosylated hemoglobin (HbA1c) and lipid profile. **Results** :It revealed that PON1 activity was significantly lower in diabetics than control ($p< 0.001$) and is lower in group III (183.6 ± 52.01) than group II (230.05 ± 59.75) . PON1 was negatively correlated to HbA1c(r = -0.540 ,$P<0.001$) . Gene and allele frequencies was significantly different in diabetics than control at 192 polymorphism ($X^2= 7.645$, $P <0.05$) but not at 55 polymorphism with QQ higher in diabetics (77.5%) and RR higher in controls (25%). In both control and diabetics QQ and MM genotypes have the lowest activity of PON1 and RR and LL genotypes have the highest activity and QR and LM genotypes have intermediate activity ,HbA1c was highest in QQ and MM genotypes, intermediate in QR and LM genotypes and lowest in RR and LL genotypes. The allelic frequency of Q and M genotypes were higher in group III than in group II and R and L genotypes were lower in group III than in group II. **Conclusion:** Paraoxonase activity is affected by PON1 genetic variability in type2 diabetic patients. The PON1 QQ and MM genotypes are associated with lower PON1 activity than RR and LL genotypes. In type 2 diabetic patients the QQ and MM genotypes are more common and associated with poor glucose control mainly in diabetic patients with vascular complication which suggest their essential roles in occurrence of diabetic vascular complications.
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Key words: PON1 gene polymorphism, diabetic complication, PON1 activity, HbA1c.

1. Introduction

The oxidative modification of low-density lipoprotein (LDL) in the artery wall is believed to be central to the pathogenesis of atherosclerosis. One such mechanism is the prevention of LDL oxidation by high-density lipoprotein (HDL). HDL appears to decrease the accumulation of lipid peroxides on LDL by a mechanism that is, at least in part, enzymatic[1].

The prevention of oxidation of LDL is largely attributable to - HDL associated enzyme – paraoxonase-1 (PON1). Besides lipid peroxides, PON has been found to hydrolyzes hydrogen peroxides, which are a major reactive species produced by the arterial wall during atherogenesis[2].

Paraoxonase-1(PON1) is part of a multi-gene family also comprising the PON2 and PON3 genes, which are also located on chromosome7[3].

The PON1 gene product is serum paraoxonase, which is expressed mainly in the liver. Serum paraoxonase circulates as sub fraction of HDL and appears to use phospholipids on both high and low density lipoprotein particles as physiological substrate [4].

Paraoxonase-1 has two genetic polymorphisms, both due to amino acid substitutions: one involving glutamine (Q genotype) and arginine (R genotype) at position 192, and the other involving leucine (L genotype) and methionine (M genotype) at position 55. These polymorphisms affect the hydrolytic activity of the PON1 isoenzymes with respect to certain substrates, such as paraoxon and lipid peroxides[1].

There is evidence that the genetic polymorphisms of PON1 least able to protect LDL against lipid peroxidation are overrepresented in coronary heart disease, particularly in association with diabetes [5].

The chronic vascular complications of diabetes are a major cause of morbidity and premature mortality. In spite of more wide-spread availability of intensive diabetes management, approximately one in three people with diabetes develop aggressive

complications and over 70% die of atherosclerosis-related diseases. Potential mediators of vascular damage may include the interrelated processes of lipoproteins abnormalities, glycation, oxidation and endothelial dysfunction. Therefore, recognition and treatment of lipoprotein related risk factors may facilitate early recognition and treatment of high risk diabetic patients [6].

The aim of the present study is to investigate paraoxonase-1 activity and its gene polymorphisms (PON1 55 & PON1 192) in patients with type 2 diabetes mellitus as well as the potential significance of these polymorphisms in the occurrence of diabetic complications.

2. Subjects and Methods:

This study was carried out at Clinical Pathology and Internal Medicine Departments, Faculty of Medicine, Zagazig University Hospitals.

Subjects: The study included 60 subjects, they give consent about the sampling they were classified into the following: Twenty apparently healthy subjects**,** their ages ranged from 38 to 68 years with a mean ± SD of 52.95±9.24 years (Group I) .Twenty patients with type 2 diabetes mellitus without vascular complications, their ages ranged from 40 to 67 years with a mean ± SD of 54.85±8.11 years(Group II) . Twenty patients with type 2 diabetes mellitus with vascular complications, Their ages ranged from 45 to 71 years with a mean ± SD of 57.55±7.10 years (Group III). They were sub-classified into Two sub- groups according to the type of vascular complications: Ten patients with micro - vascular complications Group IIIa (5 retinopathy, 3 nephropathy , 2 neuropathy) . Their ages ranged from 49 to 68 years with a mean ± SD of 56.30±6.90years, and ten patients with macro-vascular complication Group IIIb (6 coronary heart disease (CHD), 4cerebral vascular disease (CVD). Their ages ranged from 45 to 71 years with a mean ± SD of 58.80±7.44 years. These subjects had no ketoacidosis, renal failure, liver disorder, or thyroid disease, acute or chronic inflammation, or infection. Subjects taking drugs known to affect lipoprotein oxidation or lipid-lowering drug were excluded from the study.

Methods:

After overnight fasting .EDTA blood samples were taken for HbA1c and PON1 genotyping. Sera were separated for lipid profile, liver function, kidney function tests, and PON1 activity, and blood samples were collected on Na fluoride /K oxalate tubes for FPG, PPPG.

All members of this study were subjected to full history taking, thorough clinical examination, routine laboratory investigations :Fasting and post prandial plasma glucose (FPG, PPPG), Liver & kidney function tests, and Lipid profile .All previous tests (except HDL-C and LDL-C) were determined on ADVIA 1650 auto-analyzer (Siemens Medical Solutions Diagnostic, USA). HDL-C was determined using Eli-tech kit (ELI-TECH -Diagnostics, France)[7]**.** LDL-cholesterol (LDL-C) was calculated using Friedewald's equation [8]. Glycosylated haemoglobin (HbAlc) by ion exchange resin chromatography using Stanbio Glycohaemoglobin[9]. Analysis of PON1 activity using paraoxon (*O*,*O*-diethyl-*O*-*p*-nitrophenyl phosphate); Sigma-Aldrich ,Germany) as a substrate according to the method described by Mackness *et al.* [10]. Determination of PON1 genotype was conducted by PCR amplification, followed by polymorphism-specific restriction enzyme digestion and gel electrophoresis [11]. Total genomic DNA was purified from buffy coat samples using the E.Z.N.A. ™ Blood DNA Kit (Omega – biotek. Inc). Genomic DNA samples were stored at -20°C until genotyping analysis. For the polymorphism at position 192 ,sense primer 5′ TATTGTTGCTGTGGGACCTGAG 3′, Antisense primer 5′ CACGCTAAACCCAAATACATCTC 3′, For the polymorphism at position 55 ,Sense primer 5′ GAAGAGTGATGTATAGCCCCAG 3′,and Antisense primer 5′ TTTAATCCAGAGCTAATGAAAGCC 3′ were used. Amplification steps of DNA was performed by pure Taq ready –To-Go PCR Beads (Amersham Biosciences).The PCR reaction mixture contained 5 µl of template DNA ,3 µl of each primer (100 uM/L) [Opern] in a tube containing one PCR bead [200 uM of each dNTP in 10 mM Tris-HCL(pH9.0),50 mM HCL and 1.5 mM $MgCl_2$and 2.5 U Taq DNA polymerase].After denaturating the DNA for 5 min at 95 °c for 1 cycle, the reaction mixture was subjected to 46 cycles of 1 min of denaturation at 94°C, 30 sec of annealing at 61 °C, and1 min of extension at 72 °C for the polymorphism at position 192. For the polymorphism at position 55 the PCR reaction and cycling were the same as descibed above, except that 30 cycles were carried out. PCR product was digested with 8 units of restriction endonucleases Alw I and Nla III (New England Biolabs, Cambridge, MA, U.S.A.) overnight at 37°C. The digested product was separated by agarose gel electrophoresis using DNA ladder 50bp. After finishing the procedure, the gel was viewed and photographed using UV illumination for sample visualization.

At position 192, allele Q (glutamine) corresponds to the presence of non-digested fragment of 99 bp product, while allele R (arginine) corresponds to 2 digested fragments of 66 bp and 33 bp. Characteristic bands were obtained at the following molecular weights: 99 for the PON1 192QQ polymorphism; 66 and 33 for the RR

polymorphism, and 99, 66, and 33 for the QR polymorphism .

At position 55, allele L (leucine) corresponds to the presence of non-digested fragment of 170 bp, while allele M (methionine) corresponds to 2 digested fragments of 126 bp and 44 bp. Characteristic bands were obtained at the following molecular weights: 170 for the PON1 55LL polymorphism; 126 and 44 for the MM polymorphism, and 170, 126, and 44 for the LM polymorphism.

Statistical Analysis

Statistical analysis was performed with the Statistical package for social scientists "SPSS" program for windows 10 (SPSS Inc., Chicago, IL, USA). Results were expressed as mean± standard deviation (SD), Chi-square and student-t test was used for statistical comparisons between two groups of patients' parametric data. Analysis of variance (ANOVA) and least significant difference (LSD) were done to test the difference between the different studied groups. Correlation analysis was performed with Pearson correlation test. P-values below 0.05 were considered significant.

3. Results:

As regard FPG, PPPG, HbA1c ,triglycerides, total cholesterol, HDL-C, LDL-C and PON-1activity, there were high significant difference between all studied groups ($P < 0.001$),and between each two studied groups (Table1).

As regard PON1 activity there was no significant difference in PON1 activity between groups IIIa and IIIb (t=1.612, $p > 0.05$) (Table 2).

There was no significant correlation between PON1 activity and other studied parameters in controls, diabetic patients except for HbA1c in diabetic patients. where there was a significant negative correlation between PON1 activity and Hb A1C (r = -0.540, $P < 0.001$) (Table 3).

There were no significant differences in serum level of, triglycerides, total cholesterol, HDL-C, LDL-C between PON1 192,PON1 55 genotypes among both control and diabetic patients group ($P>0.05$) (not shown in tables).

As regard PON1 genotypes distributions and allele frequencies in the studied groups, the QQ genotype (Gln/Gln) was the most common in diabetic patients (77.5%) as well as in controls(45%), while the RR genotype (Arg/Arg) was the lowest one in both (5%,25%) respectively. The genotypes distributions of the PON1 192 polymorphisms was significantly different in diabetic patients compared to controls ($X^2=7.645$, $P<0.05$).The allelic frequency of Glycine192 (Q) was higher in the diabetic patients than controls (86% vs. 60%). Significant difference between the allele frequencies for the PON1 192 polymorphism was detected in diabetic patients as compared to controls ($X^2=5.175$, $P <0.05$). Also, the LL genotype (Leu/Leu) was the most common in controls (60%) , whereas the LM(47.5%) was more common than the LL genotype in diabetic patients. The genotype distribution of PON1 55 polymorphisms in diabetic patients was not statistically significant different compared to controls ($X^2=4.901$, $P >0.05$). The allelic frequency of Methionine 55 (M) was higher in the diabetic group than controls (44% vs. 22.5%) but no statistically significant difference between controls and diabetic patients was detected ($X^2=2.353$, $P>0.05$). (Table 4).

In control group, as regard PON1 activity, there was a significant difference among different PON1 192 genotypes (F= 12.166, $P <0.01$). Also, there was a significant difference in PON1 activity among different PON1 55 genotypes (F= 7.155, $P <0.01$). PON1 activity was lower in QQ,MM, intermediate in QR,LM and higher in RR,LL genotypes.(Table 5).In diabetic patients, as regard PON1 activity, there was a significant difference among different PON1 192 genotypes (F= 4.276, $P<0.05$). Also, there was a significant difference in PON1 activity among different PON1 55 genotypes (F= 3.557, $P<0.05$). PON1 activity was lower in QQ, MM, intermediate in QR, LM and higher in RR ,LL genotypes.(Table 6).

In diabetic patients, as regard HbA1cvalues, there was a significant difference among different PON1 192 genotypes (F= 3.986, $P <0.05$). Also, there was a significant difference in Hb A1c values among different PON1 55 genotypes (F= 4.346, $P <0.05$). HbA1cvalues was higher in patient with QQ,MM , intermediate in QR, LM and lower in RR,LL genotypes .(Table 7).The genotypes distributions of the PON1 192 polymorphisms was significantly different in group II compared to group III ($X^2=7.152$, $P <0.05$). The allelic frequency of Glycine 192 (Q) was higher in the diabetic group with vascular complication than diabetic group without vascular complication ($X^2=5.175$, $P <0.05$). The genotype distribution of PON1 55 polymorphisms was significantly different in group II compared to group III ($X^2=8.322$, $P <0.05$). The allelic frequency of Methionine 55 (M) was higher in the diabetic group with vascular complication than diabetic group without vascular complication ($X^2=5.125$, $P <0.05$).(Table8).

Table (1): Biochemical findings of the three studied groups

Group / Parameter	Group 1 (n = 20) Mean± SD (range)	Group II (n = 20) Mean ±SD (range)	Group III (n = 20) Mean± SD(range)	F	*P*
FPG (mg/ dl)	91.4 ± 10.57 (73 -107)	157.55±33.25* (97- 243)	168.75 ± 30.74** (117 - 213)	48.478	< 0.001
PPPG (mg/dl)	114.9 ± 11.69 (95 – 130)	242.4 ± 52.92* (163 – 365)	265.45 ± 48.74** (172 – 367)	74.257	< 0.001
Hb A1c %	4.38 ± 0.608 (3 – 5.5)	9.11 ± 0.954* (7.4 – 10.9)	10.427± 0.764**# (8.9 – 11.5)	325.58	< 0.001
Triglycerides (mg/dl)	122.2± 24.9 (81-160)	213.45 ± 56.65* (105-305)c	267.6 ± 59.12**# (152-345)	44.241	< 0.001
Total cholesterol (mg/dl)	172.3 ± 20.89 (142-214)	223.5 ± 25.98* (163-257)	250.7± 38.66**# (173-310)	36.489	< 0.001
HDL –C (mg/dl)	52.85 ± 10.43 (40-70a,b	41.85 ± 7.84* (34-60)	39.45 ± 7.07** (30-52)	13.904	< 0.001
LDL -C (mg/dl)	95.01± 23.55 (55-135.8)	138.96 ± 25.2* (82.4-173)	157.73±43.76** (79-224.8)	20.031	< 0.001
PON1Activity (U/L)	301.7± 98.74 (150-466)	230.05 ± 59.75* (121-368)	183.6 ± 52.01**# (112-320)	13.254	< 0.001

*statistical significant with group I ** statistical significant with group I # statistical significant with group II

Table (2): Comparison of mean ± SD and range between groups (IIIa) and (IIIb) as regard PON1activity (U/L).

Group / Parameter	Group IIIa (n = 10) Mean± SD (range)	Group IIIb (n=10) Mean± SD (range)	T test	
			t	*P*
PON1 Activity (U/L)	201.6± 32.22 (153-252)	165.65 ± 62.87 (112-320)	1.612	> 0.05 NS

Table (3):Correlation between PON1 activity(U/L)and other studied parameters in the control group and diabetic subjects .

Parameters / Group	Control group (n = 20)		Diabetic patients (n = 40)	
	r	*P*	r	*P*
HbA1c%	- 0.136	> 0.05	-0.540	< 0.001.H.S
FPG(mg/dl)	0.14	> 0.05	0.242	> 0.05
PPPG(mg/dl)	0.386	> 0.05	-0.089	> 0.05
Triglycerides(mg/dl)	0.245	> 0.05	-0.275	> 0.05
Cholesterol(mg/dl)	-0.087	> 0.05	0.083	> 0.05
HDL-C(mg/dl)	0.217	> 0.05	0.259	> 0.05
LDL-C(mg/dl)	-0.225	> 0.05	0.123	> 0.05

P>0.05 non-significant

Table (4): PON1 genotypes distributions and allele frequencies in the studied groups.

Groups / Parameters	Control group (n = 20)		Diabetic patients (n= 40)		X^2	*P*
	N0	%	No.	%		
PON192genotypes						
QQ	9	45	31	77.5		
QR	6	30	7	17.5	7.645	<0.05 S
RR	5	25	2	5		
Allele frequencies						
Q		60		86	5.175	<0.05 S
R		40		14		
PON55genotypes						
LL	12	60	13	32.5		
LM	7	35	19	47.5	4.901	>0.05 N.S
MM	1	5	8	20		
Allele frequencies						
L		77.5		56	2.353	>0.05 N.S
M		22.5		44		

Table (5): PON1 activity(u/l) in control group according to their PON1 192, PON1 55 genotypes.

	Control group (n=20)		
	PON1 activity(u/l) Mean± SD (range)	F	*P*
PON192genotypes QQ(n=9) QR(n=6) RR(n=5)	 242.78±66.73 (150-363) 287±86.97 (152-381) 425.4±26.23(400-466)	12.166	<0.01 S
PON55genotypes LL(n=12) LM(n=7) MM(n=1)	 348±86.18(167-466) 266.67±47.85(152-283) 150	7.155	<0.01 S

Table (6): PON1 activity(u/l) in diabetic patients according to their PON1 192, PON1 55 genotypes.

Genotypes	Diabetic patients (n=40)		
	PON1 activity(u/l) Mean± SD (range)	F	*P*
PON192genotypes QQ(n=31) QR(n=7) RR(n=2)	 193.29±54.4(112-311) 247.29±59.94 (186-368) 275±63.64(230-320)	4.276	<0.05 S
PON55genotypes LL(n=13) LM(n=19) MM(n=8)	 231.64±64.4(131-368) 206.17±53.4(126-311) 164.88±47.37(112-260)	3.557	<0.05 S

Table (7): HbA1c % in diabetic patients according to their PON1 192, PON1 55 genotypes.

Genotypes	Diabetic patients (n=40)		
	HbA1c% Mean± SD (range)	F	*P*
PON192genotypes QQ(n=31) QR(n=7) RR(n=2)	 10.011±0.955(7.9-11.5) 9.035±0.757(8.5-10.7) 8.906±1.269 (7.4-9.6)	3.986	<0.05 S
PON55genotypes LL(n=13) LM(n=19) MM(n=8)	 9.432±1.062(7.9-11.4)a 9.612±1.060(7.4-11.1)b 10.688±0.683(9.5-11.5)	4.346	<0.05 S

Table (8): PON1 genotypes distributions in group II and III.

Groups / Parameters	Group II (n = 20)		Group III (n= 20)		X^2	*P*
	N0	%	No.	%		
PON192genotypes QQ QR RR	 12 6 2	 60 30 10	 19 1	 95 5 0	7.152	<0.05 S
Allele frequencies Q R		 75 25		 97.5 2.5	5.125	<0.05 S
PON55genotypes LL LM MM	 10 9 1	 50 45 5	 3 10 7	 15 50 35	8.322	<0.05 S
Allele frequencies L M		 72.5 27.5		 40 60	5.175	<0.05 S

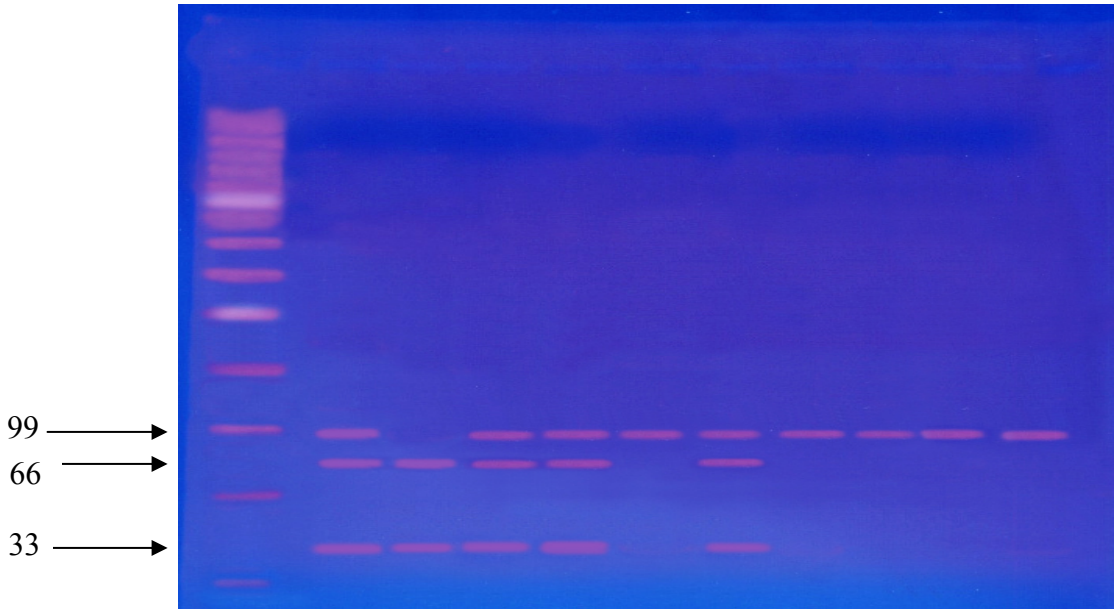

Figure (1): Agarose gel electrophoresis of the PCR products of the PON1 192 genotype in group II. Lane 1 represents 50 bp DNA marker, lanes 6,8,9,10,11 represent QQ genotype, lanes2,4,5,7 represent QR genotype, lane 3 represent RR genotype.

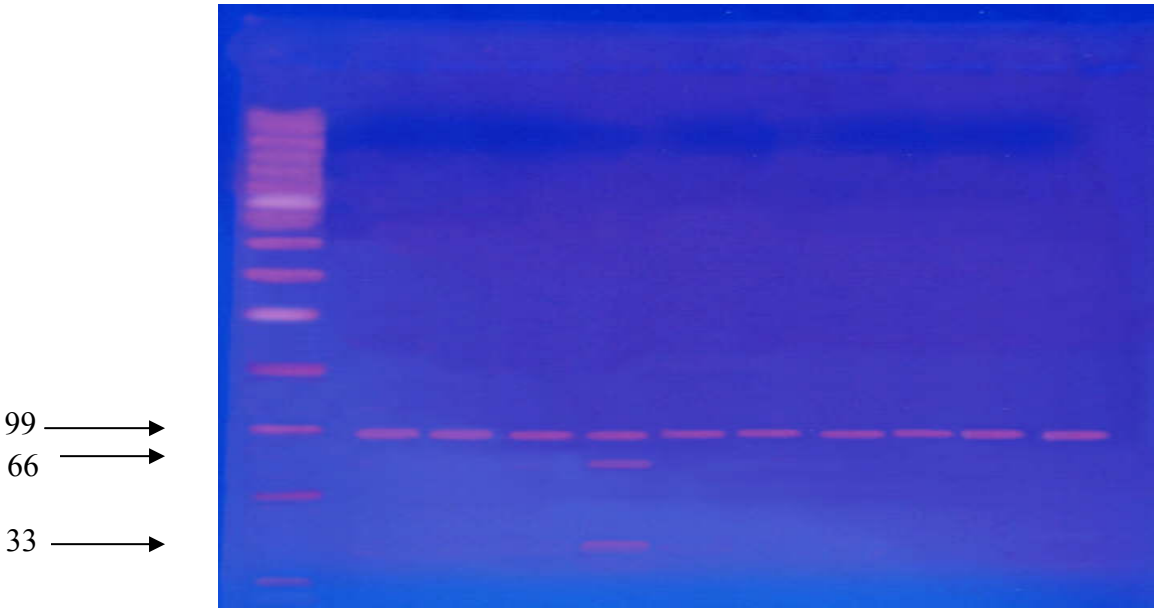

Figure (2): Agarose gel electrophoresis of the PCR products of the PON1 192 genotype in group III. Lane 1 represents 50 bp DNA marker, lanes 2,3,4,6,7,8,9,10,11 represent QQ genotype, lane 5 represent RR genotype.

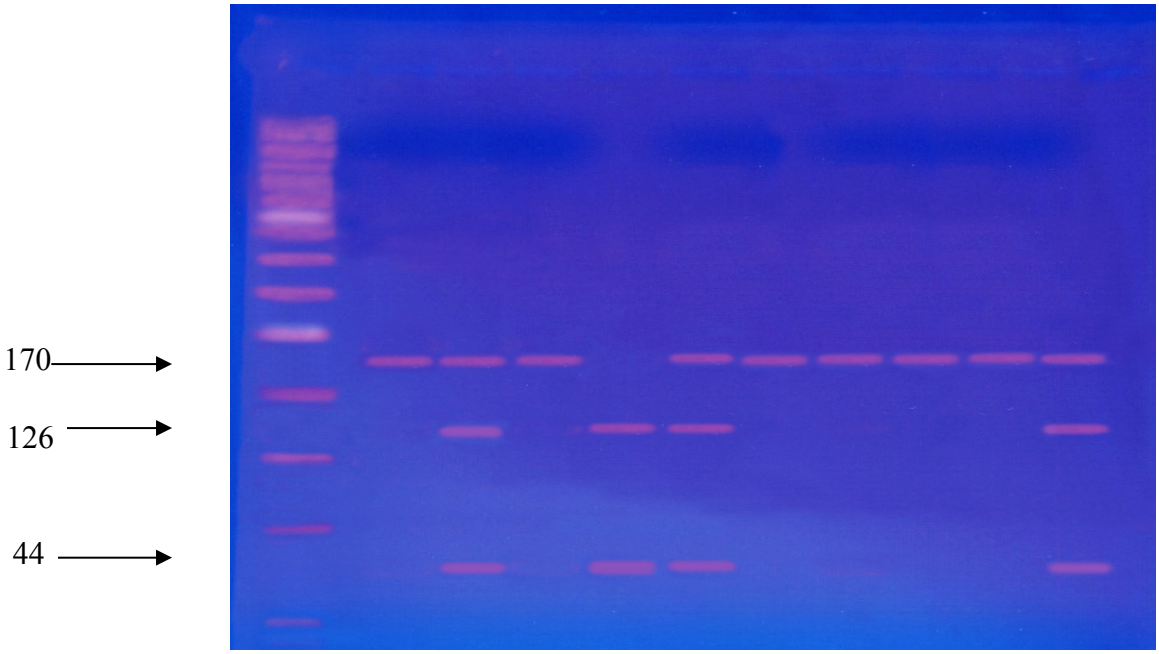

Figure (3): Agarose gel electrophoresis of the PCR products of the PON1 55 genotype in group II. Lane 1 represents 50 bp DNA marker, lanes 2,4,7,8,9,10 represent LL genotype, lanes 3,6,11 represent LM genotype, lane 5 represent MM genotype.

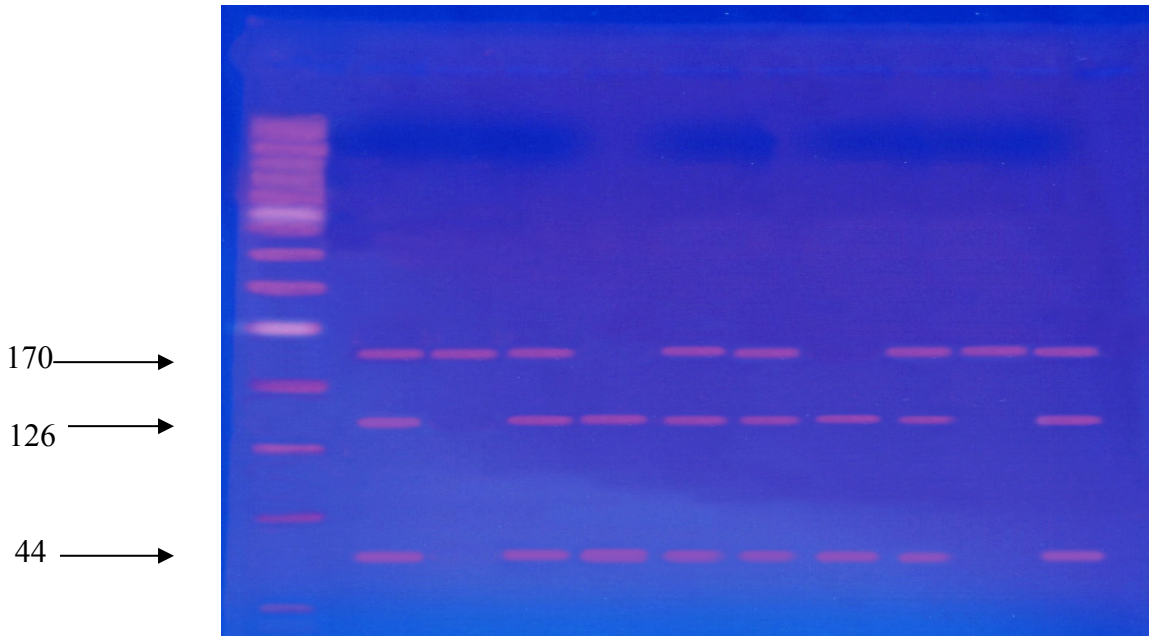

Figure (4): Agarose gel electrophoresis of the PCR products of the PON1 55 genotype in group III. Lane 1 represents 50 bp DNA marker, lanes 3,10 represent LL genotype, lanes 2,4,6,7,9,11 represent LM genotype, lanes 5,8 represent MM genotype.

4.Discussion:

It is a well established fact that diabetes is a risk factor for cardiovascular disease [12]. While microvascular complications of diabetes include nephropathy and retinopathy, macrovascualr complications resulting in atherosclerotic cardiovascular disease such as coronary artery disease, cerebrovascular disease and peripheral vascular disease which are the leading cause of death in the diabetic population [13].

Paraoxonase 1 is synthesized and secreted by the liver, it is a HDL associated enzyme capable of inhibition of atherosclerosis initiated by oxidatively modified LDL[2].

In this study PONI activity was significantly lower in diabetic patients compared to the control group ,and the diabetic group with vascular complications had a significantly lower level than those without vascular complications and no significant difference between subgroups with micro and macro- vascular complications. Also PONI activity was negatively correlated with HbA1cbut not correlated with serum lipids. These results are in agreement with **Sakai *et al.*** [14]**; Pfolil *et al.*** [15]; **Inoue *et al.*** [16] and **Mackness *et al.*** [1].

The reduced PONI activity could be explained by that PONI is bound by HDL in lesser extent in diabetic patients as compared to healthy persons and its activity is then poorly stabilized [17].

The reduced activity of PONI may predict its antioxidant properties which may take part in the development of vascular complications in diabetes mellitus[18]. PONI activity may be partially inactivated in the presence of oxidative stress as probably occurs in type 2 diabetes mellitus[19]. Low activity of PONI could explain the increased lipid peroxidation in this disease[20]. The negative correlation between PONI activity and HbA1c in diabetic patients coincide with **Flekac *et al.*** [21].The low enzyme activity is caused by glycation of the PONI protein than by reduced synthesis [22]. Glycation of HDL or direct glycation of PONI in HDL as occurs in diabetes may result in detatchment of PONI itself from HDL and PONI inactivation [23]. The non significant correlation between PONI activity and serum lipids is consistent with earlier findings of **Abbott *et al.*** [24]. The nonsignfiicant difference in serum lipids among PONI 192 and 55 genotypes was reported by **Odawara *et al.*** [25] and **Mackness *et al.*** [1]

Paraoxonase-1 has two genetic polymorphisms, both due to amino acid substitutions: one involving glutamine (Q genotype) and arginine (R genotype 192, and the other involving leucine (L genotype) and methionine (M) genotype at position 55. The R genotype 192 is more active than Q 192 and L55 has higher activity than M55 [26,27]**.**

In this study in 192 QQ polymorphism was the most common in both diabetics and control (77.5%,45%) respectively and 192 RR was the lowest in both diabetics and control group (5%,25%) respectively. Gene frequency at 192 polymorphism and allele frequency(Q) were significantly different in diabetics (86%)from control (60%). At 55 polymorphism gene frequency and allel frequency are not significantly different in diabetics from control. This results go hand in hand with **Mackness *et al.*** [28] and **Flekac *et al.*** [21]**, Agachan *et al.*** [29].

PONI gene polymorphism may influence variability of the enzyme activity and an association between cardiovascular disease and PONI gene polymorphism has been reported in diabetes mellitus [30]. Low PONI activity decreases ability to prevent lipid peroxide formation with consequent acceleration of the oxidant stress .Overproduction of the reactive oxygen species in diabetic patients may be due to chronic hyperglycemia, hyperinsulinemia, elevated fatty acids and dyslipidemia [31].

In this work the significant difference in PONI activity at 192 genotypes, PONI activity was lower in QQ, intermediate in QR and higher in RR genotypes in diabetic patients and in controls. These results are in accordance with **Agachan *et al.*** [32] and **Ikeda *et al.*** [33]. **Gaidukov *et al.*** [34] reported that Q192 alloenzyme exhibited lowerstability, lipolactonase and modulatory effect on macrophage cholesterol efflux. **Bhattacharyya *et al.*** [35] found that R 192 polymorphism individuals have higher serum PONI activity and reduction in prevalent coronary artery disease.

The significant difference in PONI 55 genotypes in this study in PONI activity was lower in MM, intermediate in LM and higher in LL genotypes in diabetic patients and controls. These results are in agreement with those of **Garin *et al.*** [36]

The HbA1c results was higher in QQ, MM genotypes than in RR, LL genotypes respectively and patients with QR, LM genotypes had intermediate diabetic control. These results are in agreement with **Mackness *et al.*** [26] and **Flekac *et al.*** [21] who concluded that QQ and MM genotypes in diabetes is associated with poorer glucose control.

This study found that the genotype distribution of 192 polymorphism and 55 genotype are significantly different in diabetic group with vascular complications than those without vascular complications in agreement with **Altuner *et al.***.[37] .Also the allelic frequencies of both 192(Q) and 55 (M) were higher in the diabetic group with vascular complications compared to diabetic group without vascular complications. Q (97.5 VS.75%), M(60%VS.27.5%) .Also there were a lower frequency of R allele(2.5% VS.25%) and L allele(40%VS.72.5) in those with vascular complications than in group without vascular complication this was hand in hand with **Agachan *et al.*** [29]**.**

These results were in agree with **Flekac *et al.*** [21]. who support that the association of MM and QQ genotypes with poorer diabetic control and more decreased enzyme activity in angiopathy relates to the

assumption that L and R carriers might be better protected against atherosclerosis.

It is probable that Pon1 activity may be partially inactivated in the presence of oxidative stress as occurred with type 2 diabetes mellitus. In both MM and QQ genotypes had higher thiobarbituric acid reactive substances(TBARS) and lower glutathione (GSH) level, whereas genotypes of PONI RR and PONI 55 LL alleles have lower TBARS and higher GSH levels and have protective effects against oxidative stress. [38]

Conclusion

Paraoxonase activity is affected by PON1 genetic variability in type2 diabetic patients and controls. The PON1 192 RR and 55 LL genotypes are associated with higher PON1 activity than QQ and MM genotypes. Higher prevalence of QQ and MM genotypes in diabetes is associated with poorer glucose control and more common in diabetic group with vascular complications. This may suggest their essential role in occurrence of diabetic vascular complications.

Conflict of interests: none.

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References

1-Mackness B., Durrington PN., Abuashia B., Boulton AJ. and Mackness MI. (2000): Low paraoxonase activity in type II diabetes mellitus complicated by retinopathy. *Clin. Sci. 98: 355-363.*

2-Sentí M., Tomás M., Fitó M., Weinbrenner T., Covas MI. and Sala J.(2003): Antioxidant paraoxonase 1 activity in the metabolic syndrome. *J Clin Endocrinol Metab., 88:5422-5426.*

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

4-Hegele RA., (1999):Paraoxonase genes and disease. *Ann Med. 31(3): 217-224.*

5-Durrington PN., Mackness B. and Mackness MI.(2001): Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol., 21:473*

6-Jenkins AJ., Best JD., Klien RL. and Lyonss TJ.(2004):Lipoproteins, glycoxidation and diabetic angiopathy. *Diabetes Metab Res Rev 20(5):349-368.*

7-Burstein M., Scholnick HR., and Morfin R.,(1970):Rapid method of isolation of lipoproteins from human serum by precipitation with polyanion. J Lipid Res., 11 (6): 583-595.

8-Friedewald WT., Levy RI. and Fredrickson DS.(1972): Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem., 18: 499-502.*

9-Svendsen P.(1980):Methods used for measuring FHb concentration. *Diabetologia 19:130-136.*

10-Mackness MI., Harty D., Bhatnagar D., Winocour PH., Arrol S. and Ishola M.(1991): Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. *Atherosclerosis 86:193-199.*

11-Humbert R., Adler DA., Disteche CM., Hassett C., Omiecinski CJ. and Furlong CE. (1993): The molecular basis of the human serum paraoxonase activity polymorphism .*Nat Genet 3: 73-76.*

*12-L*aakso M.(1999): Hyperglycemia and cardiovascular disease in type 2 diabetes. *Diabetes 48: 937-942.*

13-American diabetes association. (2002): Summary of revisions for the 2002 clinical practice recommendations additions to the clinical practice recommendations. *Diabetes Care 25:S3.*

14-Sakai T., Matsuura B. and Onji M.(1998): Serum paraoxonase activity and genotype distribution in Japanese patients with diabetes mellitus. *Intern Med., 37:581-584.*

15-Pfohl M., Koch M., Enderle MD., Kühn R., Füllhase J. and Karsch KR.(1999): Paraoxonase 192 Gln/Arg gene polymorphism, coronary artery disease, and myocardial infarction in type 2 diabetes. *Diabetes 48:623-627.*

16-Inoue M., Suehiro T., Nakamura T., Ikeda Y., Kumon Y. and Hashimoto K.(2000):Serum arylesterase/ diazoxonase activity and genetic polymorphisms in patients with type 2 diabetes. *Metabolism, 49: 1400-1405.*

17-Baum L., Ng HK., Woo KS., Tomlinson B., Rainer TH., Chen X., Cheung WS., Chan DK., Thomas GN., Tong CS. and Wong KS.(2006): Paraoxonase 1 gene Q192R polymorphism affects stroke and myocardial infarction risk. *Clin Biochem, 39: 191-195.*

18-Dantoine T., Debord J., Merle L. and Charmes JP.(2003): From organophosphate compound toxicity to atherosclerosis: role of paraoxonase 1. *Rev Med Interne, 24: 436-442*

19-Aviram M., Rosenblat M. and Billecke S. (1999):Human serum paraoxonase (PON1) is inactivated by oxidised low density lipoprotein

and preserved by antioxidants. *Free Radicals Biol. Med. 26: 89*

20-Letellier C., Durou MR., Jouanolle AM., Le Gall JY., Poirier JY. and Ruelland A. (2002): Serum paraoxonase activity and paraoxonase gene polymorphism in type 2 diabetic patients with or without vascular complications. *Diabetes Metab. 28 297-304.*

21-Flekac M., Škrha J., Zidkova K., Lacinova Z. and Hilgertova J.(2008): Paraoxonase 1 gene polymorphisms and enzyme activities in diabetes mellitus. *Physiol. Res. 57: 717-726.*

22-Hedrick CC., Thorpe SR., Fu MX., Harper CM., Yoo J. and Kim SM.(2000): Glycation impairs high-density lipoprotein function. *Diabetologia 43:312-320.*

23-Karabina SA., Lehner AN., Frank E., Parthasarathy S. and Santanam N. (2005): Oxidative inactivation of paraoxonase - implications in diabetes mellitus and atherosclerosi s. *Biochim Biophys Acta, 1725:213-221.*

*24-A*bbott CA., Mackness MI., Kumar S. , Boulton AJ. and Durrington PN. (1995): Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins Arterioscler Thromb Vas Biol 15:1812-1818

25-Odawara M., Tachi Y. and Yamashita K.(1997): Paraoxonase polymorphism (Gln192-Arg) is associated with coronary heart disease in Japanese non-insulin-dependent diabetic patients. *J Clin Endocrinol Metab., 82: 2257-2260.*

26-Billecke S., Draganov D. and Counsell R. (2000): Human serum paraoxonase (PON1) isozymes Q and R hydrolyze lactones and cyclic carbonate esters. *Drug Metab. Dispos. 28, 1335–1342.*

27-Blatter-Garin MC., James RW. and Dussoix P. (1997):Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. *J. Clin. Invest. 99: 62–66***.**

28-Mackness B., Durrington PN., Boulton AJM., Hine D. and Mackness MI.(2002): Serum paraoxonase activity in patients with type I diabetes comparedto healthy controls. *Eur J Clin Invest., 32:259-64.*

29-Agachan B., Yilmaz H., Ergen HA., Karaali ZE. and Isbir T. (2005): Paraoxonase (PON1) 55 and 192 Polymorphism and Its Effects to Oxidant-Antioxidant System in TurkishPatients with Type 2 Diabetes Mellitus . *Physiol. Res. 54: 287-293.*

30-Fortunato G., Rubba P., Panico S., Trono D., Tinto N., Mazzaccara C., Michelem D., Iannuzzi A., Vitale DF., Salvatore F. and Sacchetti L.(2003): A paraoxonase gene polymorphism, PON 1 (55), as an independent risk factor for increased carotid intima-media thickness in middle-aged women. *Atherosclerosis 167 141-148.*

31-Maritim AC., Sanders RA. and Watkins JB.(2003): Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol.,17: 24-38, 2003.*

32-Agachan B., Yilmaz H., Karaali Z. and Isbir T. (2004): Paraoxonase 55 and 192 polymorphism and its relationship to serum paraoxonase activity and serum lipids in Turkish patients with non-insulin dependent diabetes mellitus.*Cell. Biochem. Funct. 22: 163-168.*

33-Ikeda Y., Suehiro T., Inoue M., Nakauchi Y., Morita T. and Arii K.(1998):Serum paraoxonase activity and its relationship to diabetic complications in patients with non-insulin-dependent diabetes mellitus. *Metabolism 47:598-602.*

34-Gaidukov L., Rosenblat M. and Aviram M.(2006): The 192R/Q polymorphs of serum paraoxonase PON1 differ in HDL binding, lipolactonase stimulation, and cholesterol efflux.*J Lipid Res 47(11): 2492-2502.*

35-Bhattacharyya T., Nicholls SJ. and Topol EJ. (2008): Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. *JAMA. 299(11): 1265-1276*

36-Garin MC., James RW., Dussoix P., Blanche H., Passa P. and Froguel P. (1997): Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. *J Clin Invest., 99:62-66.*

37- Altuner D., Suzen S.H., Ates I., Koc G.V., Aral Y. *,and Karakaya A.(2011).*: Are PON1 Q/R 192 and M/L 55 polymorphisms risk factors for diabetes complications in Turkish population? Clin. Biochem., 44 ,372-6

38- ErgunM.A., Yurtcu E., Demirci H., IlhanM.N., BarkarV. *,Yelkin I.,Menevse A* .(2001). PON1 55 and 192 gene polymorphisms in type 2 diabetes mellitus patients in a Turkish population Biochem. Genet., 49 , 1–8.