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RESEARCH ARTICLE

ASSOCIATION BETWEEN PARAOXONASE 1 ALLELIC FREQUENCY DISTRIBUTION, CRUDE ODDS RATIOS, AND THE RISK OF CARDIOVASCULAR DISEASES IN A MOROCCAN POPULATION

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Abstract

Aim : Paraoxonase 1 (PON1) an antioxidant enzyme associated with high density lipoprotein (HDL) is expressed in the Islet of Langerhans and may play a protective role against oxidative stress. This study inves tigated the relationship between the PON1 phenotype, allelic frequency distribution and the risk of cardiovascular disease complications.

Methods : Three hundred subjects aged between 40-80 years including healthy controls, diabetic patients, and chronic renal failure/hemodialys is patients, were enrolled and divided into three groups of 100 subjects each. Participants were further stratified by age into middle-aged 40-59 years, (n=147), and elderly subjects 60-79 years (n = 153). Plasma malondialdehyde levels were measured by HPLC using thiobarbituric acid (TBA) derivatization with fluorescence detection. Vitamin E (α-tocopherol) concentration was determined by HPLC with electrochemic al detection.

Results : PON 1 allelic frequencies in healthy, diabetic and hemodialys is subjects were as respectively : Q alleles 86.62%, 85.46% and 79.78%; R alleles 13.37%, 14.53% and 20.22%. In hemodialysis patients, the crude odds ratio (OR) comparing the QQ with the RR phenotypes was 1.97 [95 % (CI): 0.63-6.21]. In diabetic patients, the OR comparing the QQ with the QR phenotypes was 1.37 [95% CI: 0.62-3.04]. PON1 activity was significantly higher in QR+RR carriers than in QQ carriers among diabetic and hemodialysis patients ($p<0.001$ for both groups).

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The vitamin E/Total Cholesterol ratio was significantly reduced in diabetic QQ carriers ($p<0.05$) and hemodialysis QQ carriers ($p<0.05$) compared with healthy subjects.
Conclusion: These findings indicate differences in PON1 phenotype distribution and oxidative stress markers among healthy, diabetic and hemodialysis subjects. However, the observed odds ratios were not statistically significant and therefore the results should be considered exploratory and hypothesis-generating.

Introduction:-

Diabetes and renal failure are increasingly prevalent health problems in developing countries, including Morocco and represent major risk factors for cardiovascular disease and myocardial infarction [1-5]. Paraoxonase 1 (PON1) is an antioxidant enzyme associated with HDL that hydrolyzes lipid peroxides in LDL and atherosclerotic lesions [6-7]. Three paraoxonase isoforms have been identified (PON1, PON2 and PON3), although only PON1 exhibits true paraoxonase activity. Oxidized LDL plays a central role in the cardiovascular complications associated with diabetes and hemodialysis [7]. Reduced PON1 activity in diabetic and hemodialysis patients may contribute to increased susceptibility to lipid peroxidation, partly due to hyperglycaemia and the dialysis process [5-12]. Therefore, elevated paraoxonase activity may provide protective cardiovascular effects in these populations [2,15]. One of the factor determinants of PON1 activity is the Q192R genetic polymorphism, which gives rise to two isoforms: Q (low Paraoxonase activity) and R (high Paraoxonase activity) [8, 9]. The R allele has been associated with increased risk factor for cardiovascular disease and diabetic or hemodialysis patients carrying this allele may be at greater risk of cardiovascular complications [2, 7-9]. Previously, we determined the distribution of PON1 phenotypes in a Moroccan population [2]. The present study aimed to investigate the association between alterations in PON1 phenotype distribution and the risk of cardiovascular disease in healthy subjects, diabetic patients, and hemodialysis patients.

Materials and Methods:-

Subjects:-

Three hundred subjects aged 40-80 years were enrolled in this study. Participants were recruited from the Biomedical center of the Pasteur Institute of Casablanca for routine medical evaluation. Before, they went at the Cardiology Service of the University Hospital Center Ibn Rochd in Casablanca, Morocco and received instrumental examinations, including coronary angiograms and echocardiograms. Cardiovascular disease was defined as a documented history of coronary artery disease, myocardial infarction, angina, coronary revascularization, ischemic stroke, or peripheral arterial disease confirmed from medical records and cardiology evaluations.

Diabetic patients (n = 100) were recruited from local clinical centers, whereas hemodialysis patients (n = 100) were recruited from hemodialysis centers in Casablanca. Participants were divided into three groups according to health status for case control study: healthy control (n=100), diabetic patients (n = 100), hemodialysis patients (n = 100). Pregnant and breastfeeding women were excluded, and only one individual per family was included among diabetic and hemodialysis patients.

Healthy subjects were nonsmokers and nondrinkers with no clinically apparent cardiovascular disease, normal renal and liver function and no clinical or laboratory evidence of diabetes or inflammation (hs-CRP < 3; fasting blood glucose < 6.1 mmol/l). None were taking medication or oral antioxidant supplements.

Among diabetic patients, 75 % were hypertensive, 23 % had a family history of diabetes, and most were receiving statin therapy. The mean duration of was 4.33 ± 2.42 years, and diabetic nephropathy was present in 22% of patients. One subject had previously undergone surgery for disc herniation, and another had a solitary kidney. Cardiovascular disease was present in 40 % of diabetic patients, and three patients were treated with insulin.

Hemodialysis subjects received standard unfractionated heparin administered in fractional doses every hour during dialysis sessions. The delivered dialysis dose corresponded to a Kt/V is 1.2. Patients underwent hemodialysis for 4 hours, three times per week, using bicarbonate-based dialysate. Hemophane membranes were the most used membranes. Among the hemodialysis subjects, 55 % were hypertensive and 15 % were receiving statin therapy.

The mean duration of hemodialysis treatment was 53 months. One subject had previously undergone kidney transplantation, another had valvular heart disease, and one subject had a solitary kidney. The study protocol was accepted by the ethics committee of the Faculty of Medicine and Pharmacy of Casablanca, and all participants provided written informed consent prior to enrollment.

All subjects underwent a physical examination and completed a self-administered questionnaire which is validated by an ad hoc committee formed. This questionnaire collected information on demographic characteristics, participants' health status, family history of diabetes, hypertension and cardiovascular diseases. It also included information regarding the duration of diabetes and hemodialysis treatment.

Body mass index (BMI) was calculated from weight and height measurements. Blood pressure was measured twice on the right arm in supine position after 5-minutes rest period to determine hypertension according to the current hypertension guidelines. Measurements were performed under quiet conditions and in the absence of emotional stress.

Blood and urine collection and lipid profile measurements :-

After a 12-hour overnight fast, 80 mL of blood were collected into sodium heparin- or EDTA-containing tubes. Plasma was separated by centrifugation (3000 x g, for 10 min) and serum aliquots were stored at – 80 º C until analysis. Microalbuminuria was in two consecutive 24-h urine samples using a BN 100 nephelometer system (Dade Behring, Germany). Serum urea and creatinine levels were assessed before each dialysis session in hemodialysis patients. Serum total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and HbA1c were measured using automated enzymatic assays (Kodak, Ektachem USA System). Serum total cholesterol levels were determining by measuring absorbance at 550 nm.

Paraoxonase activity:-

PON1 activity was determined using paraoxon (O,O-diethyl-O-p-nitrophenylphosphate; Sigma) as the substrate by measuring the increase in absorbance at 412 nm resulting from the formation of 4-nitrophenol, as previously described [2]. Briefly, 50 µl of serum were added to 1 ml of Tris-HCl buffer (100 mmol/l, pH 8.0) containing 2 mmol/l CaCl2 and 5.5 mmol/l paraoxon [9, 11, 15, 16, 17], and the reaction was monitored at 25 °C. Enzymatic activity was calculated using a molar extinction coefficient 17,100 M^{-1} cm^{-1}. One unit of paraoxonase activity was defined as 1 nmol of 4-nitrophenol generated per minute under the assay conditions.

Arylesterase assay:-

Arylesterase activity was measured spectrophotometrically using phenylacetate as substrate in 20 mM Tris/HCl pH 8.0 [16]. The increase in absorbance was monitored at 270 nm, and enzyme activity was calculated using a molar extinction coefficient of 1310 M^{-1} cm^{-1}. One unit (U) corresponded to 1 µmol phenylacetate hydrolyzed per minute [6, 18].

Paraoxonase phenotype distribution:-

The PON1 Q192R polymorphism determines two isoforms: Q (low activity) and R (high activity) [8]. PON1 phenotype was determined using the dual substrate method based on the. ratio of salt-stimulated paraoxonase activity (1 mol/l NaCl) to arylesterase activity. Subjects were classified as QQ (ratio < 3.0), QR (ratio 3.0-7) and RR phenotype (ratio > 7.0) [10, 11]. PON1 status was inferred from enzymatic phenotype determination rather than direct molecular genotyping. The dual-substrate method has been widely used as a surrogate approach for estimating the PON1 Q192R phenotype, although some degree of phenotype misclassification cannot be excluded.

Plasma vitamin E measurement:-

Plasma vitamin E was analyzed as α-tocopherol in frozen samples. Briefly, thawed plasma was mixed with an equivalent volume of ethanol (100 µL) and tocopherol were extracted in hexane (500 µL). Plasma α-tocopherol were resolved on a Sephasil reverse-phase High-Performance Liquid Chromatography (HPLC) column (C_{18}, 5 µm particles, 25 x 0.46 cm i.d; Pharmacia Biotech, Piscataway, NJ), using a methanol-ethanol-isopropanol (88:24:10, v:v:v) mobile phase containing 20 mM lithium perchlorate and a flow rate of 1 mL/min. Quantification was performed using electrochemical (ESA Coulochem II, 50-10A analytical cell) and UV detection at 292 nm. Tocopherol acetate was used as internal standard [2]. Inter-assay variability is between 1.7 % and 9.7 % and reproducibility is between 5.8 % and 9.7 %.

Thiobarbituric acid-reactive substances (TBARS) formation:-

TBARS, mainly malondialdehyde (MDA), were assayed by HPLC as described by Agarwal and Chase. The column was a HP hypersil 5 µm ODS 100 mm × 4.6 mm with a 5 µm ODS guard column and the mobile phase was a methanol-buffer (40:60, v/v). The fluorescence detector was set at an excitation wavelength of 515 nm and an emission wavelength of 553 nm. Samples of plasma were treated with the antioxidant (BHT) and heat derivatized at 100 °C for 1 h with thiobarbituric acid at an acidic pH. Samples were extracted with n-butanol and 10 µl volume of

the extract were injected [2, 19]. Inter-assay variability is between 2.52 % and 9.27 % and reproducibility is between 5.96 % and 9.11 %.

Statistical analysis:-

Statistical analyses were performed using SAS for windows version 6.11. Data are presented as mean +/- standard deviation of number cases. Comparisons between groups were performed using unpaired t-tests or ANOVA. Associations between variables were assessed using regression and Spearman correlation analyses. A two sided p value <0.05 was considered statistically significant. Hardy-Weinberg equilibrium was assessed using a χ^2 goodness-of-fit test. Because the present analyses were exploratory in nature, no formal correction for multiple comparisons was applied. Consequently, statistically significant findings should be interpreted with caution because of the potential increase in type I error.

Results:-

Baseline data:-

In the overall study population, MDA concentrations tended to be higher in carriers of the PON1 QR + RR phenotypes compared with carriers of the PON1 QQ phenotype ($p = 0.08$). MDA tended to be higher in PON1 QR carriers than in PON1 RR carriers, although the difference did not reach statistical significance ($p = 0.08$). In contrast, subjects with the PON1 QR phenotype had significantly higher MDA concentrations than PON1 QQ

carriers ($p < 0.05$) (Table 1). Among PON1 QQ carriers, MDA levels were significantly higher in diabetic subjects ($p<0.05$) and hemodialysis subjects ($p<0.01$) compared with healthy subjects with the same phenotype. Similarly, diabetic and hemodialysis subjects carrying the PON1 QR phenotype had higher MDA levels than healthy PON1 QR carriers ($p<0.05$). Among PON1 RR carriers, MDA levels were increased in diabetic and hemodialysis subjects compared with healthy RR carriers, but these differences were not statistically significant.

Paraoxonase activity was significantly higher in subjects carrying the PON1 QR or RR phenotypes compared with PON1 QQ carriers ($p<0.001$). Indeed, arylesterase activity was significantly lower in carriers of the PON1 R allele (RR and QR) compared with carriers of the PON1 Q allele ($p<0.01$). Furthermore, arylesterase activity was significantly lower in PON1 RR carriers than in PON1 QR carriers ($p<0.01$) and in combined PON1 QR+RR carriers ($p<0.05$). Consistently, arylesterase activity was significantly higher in PON1 QQ carriers compared with combined PON1 QR + RR carriers ($p<0.001$) (Table 1).

Paraoxonase ($p<0.05$) and arylesterase ($p<0.05$) activities were significantly lower in the hemodialysis subjects across all three PON1 Q192R phenotypes compared with healthy subjects. In contrast, non-significant differences in paraoxonase activity were observed between healthy subjects and the diabetic patients for any of the three PON1 Q192R phenotypes, although a slight increase in serum PON1 activity was noted in diabetic patients. Paraoxonase ($p<0.05$) and Arylesterase ($p<0.01$) activities also differed significantly among the three PON1 Q192R phenotypes in both the diabetic and hemodialysis subjects. In healthy subjects, carriers of the PON1 QQ phenotype had significantly lower paraoxonase activity compared with QR + RR carriers ($p<0.001$). Similarly, paraoxonase activity was significantly higher in QR+RR carriers than in QQ carriers among diabetic ($p<0.001$) and hemodialysis patients ($p<0.001$).

Comparison of Vitamin E/Total cholesterol ratio in the overall study population according to the PON1 Q192R polymorphism showed a significant decrease in PON1 QR carriers when compared with PON1 QQ carriers ($p<0.05$). In contrast, the Vitamin E/total cholesterol ration was significantly lower in PON1 QQ carriers compared with combined PON1 QR + RR carriers ($p<0.05$). However, no significant difference in the vitamin E/total cholesterol ratio was observed between PON1 QQ and PON1 RR carriers ($p = 0.10$) (Table 1). Vitamin E levels did not differ significantly among subjects according to the PON1 Q192R polymorphism; however, the Vitamin E/total cholesterol was significantly influenced by the PON1 phenotype (Table 1). Compared with healthy subjects carrying the PON1 QQ phenotype, the Vitamin E/total cholesterol ratio was significantly lower in diabetic ($p<0.05$) and hemodialysis subjects ($p<0.05$) carrying the same phenotype.

A significant decrease in the Vitamin E/total cholesterol ratio was observed in hemodialysis subjects carrying the PON1 QR phenotype compared with healthy QR carriers ($p<0.05$). Similarly, hemodialysis subjects carrying the PON1 RR phenotype had a significantly lower ratio Vitamin E/total cholesterol ratio than the healthy RR carriers ($p < 0.05$). Interestingly, the vitamin E/total cholesterol ratio was significantly decreased in hemodialysis subjects

carrying the combined PON1 QR + RR phenotypes compared with healthy QR+RR carriers ($p<0.01$). However, comparisons between PON1 QQ carriers and combined PON1 QR + RR carriers within healthy ($p = 0.14$), diabetic ($p = 0.32$), and hemodialysis subjects ($p = 0.10$) showed no significant differences in the Vitamin E/total cholesterol ratio.

Table 1: Baseline characteristics (mean ± SD) according to PON1 Q/R in whole subjects (n = 300)

Parameters	QQ (n=195)	QR (n=48)	QR+ RR (n=66)	RR (n=18)
Men (%)	58.46	46.93	46.97	47.05
Mean age, years	57.82 ± 11.59	57.66 ± 11.72	57.30 ± 11.82	56.33 ± 12.38
GJ (g/L)	1.50 ± 1.00	1.52 ± 0.60	1.53 ± 0.64	1.57 ± 0.83
TG, (mmol/L)	1.50 ± 1.04	2.34 ± 1.75***	2.33 ± 1.74***	1.37 ± 0.55
Serum MDA (μM)	7.43 ± 3.83	8.76 ± 4.61*	8.26 ± 4.36	7.04 ± 3.48
PON1 activity (U/mL)	38.68 ± 47.95	110.45 ± 119.40***	113.67 ± 131.35***	118.09 ± 147.87***
ARE activity (U/mL)	38.28 ± 34.24	23.72 ± 25.21**	19.47 ± 23.02***¶	8.14 ± 9.14**++
Vit. E (μM)	18.67 ± 8.52	18.66 ± 7.13	18.57 ± 7.63	18.40 ± 8.93
Vit.E/TC ratio (μmol/mmol)	4.66 ± 2.42	3.78 ± 1.83*	3.79 ± 1.95*	3.80 ± 2.21

Values are mean ± SD., unless indicated otherwise. The unpaired student t-test was applied. Significance was calculated in comparison to QQ, QR, QR + RR, RR carriers : *$p<0.05$, **$p<0.01$, ***$p<0.001$ and in comparisons between QQ and RR carriers ++$p<0.01$, and in comparison between QQ and QR+ RR carriers ¶$p<0.05$

In diabetic patients, the crude OR comparing the PON1 QQ variant with the PON1 QR variant was 1.37 (95% CI: 0.62-3.04). In contrast, the crude OR comparing the PON1 QQ and PON1 RR variants was 0.84 (95% CI: 0.22-3.25). Among hemodialysis patients, the crude ORs comparing the PON1 QQ variant with the PON1 QR and PON1 RR variants were 1.52 (95% CI: 0.69-3.35) and 1.97 (95 % CI: 0.63-6.21).

The observed allelic frequency distribution of PON1 showed Q allele frequencies of 86.62%, 85.46%, and 79.78%, for R allele frequencies of 13.37%, 14.53%, and 20.22% in healthy, diabetic and hemodialysis patients, respectively. Our results followed Hardy-Weinberg equilibrium for healthy (H-W = 0.93), diabetic (H-W = 0.96) and hemodialysis subjects (H-W = 0.90).

The crude ratio OR1 comparing the frequency of the PON1 risk allele R between heathy and diabetic subjects was 1.10 (95% CI: 0.49-2.45). Similarly, the OR2 comparing healthy and hemodialysis subjects was 1.64 (95% CI: 0.77-3.49) (Table 2).

Table 2 : Paraoxonase 1 allelic frequency distribution in healthy, diabetic and hemodialysis subjects

Allelic frequency	Healthy subjects	Diabetic subjects	Hemodialysis subjects	OR1 (95%IC)	OR2 (95%IC)
Q	86.62 ± 2.59%	85.46 ± 2.69%	79.78 ± 3%	1	1
R	13.37 ± 2.59%	14.53 ± 2.69%	20.22 ± 3%	1.10 (0.49 - 2.45)	1.64 (0.77 - 3.49)

The distribution of alleles frequencies of each polymorphism was compared between case and control subjects using χ^2 test. OR1 was calculated for diabetic versus healthy subjects, whereas OR2 was calculated for hemodialysis versus healthy subjects.

Table 3 : Paraoxonase 1 allelic frequency distribution by gender in healthy, diabetic and hemodialysis subjects

	Allelic frequency	Healthy subjects	Diabetic subjects	Hemodialysis subjects	OR1 (95%IC)	OR2 (95%IC)
Male	Q	90.69 ± 3.13%	90.54 ± 3.40%	81.54 ± 3.40%	1	1
	R	9.30 ± 3.13%	9.46 ± 3.40%	18.46 ± 3.40%	1.02 (0.39-2.64)	2.21 (0.95-5.13)
Female	Q	83.72 ± 3.98%	81.63 ± 3.91%	75 ± 6.24%	1	1
	R	16.28 ± 3.98%	18.37 ± 3.91%	25 ± 6.24%	1.16 (0.56-2.41)	1.71 (0.85-3.44)

The distribution of alleles frequencies of each polymorphism was compared between case and control subjects using χ2 test. OR1 was calculated for diabetic versus healthy subjects, whereas OR2 was calculated for hemodialysis versus healthy subject

The gender-specific distribution of PON1 allelic frequencies in men showed Q allele frequencies of 90.69 ± 3.13%, 90.54 ± 3.40%, and 81.54 ± 3.40% and R allele frequencies of 9.30 ± 3.13%, 9.46 ± 3.40%, and 18.46 ± 3.40% in healthy, diabetic and hemodialysis subjects, respectively (Table 3). Our results followed Hardy-Weinberg equilibrium for healthy (H-W = 0.98), diabetic (H-W = 0.91) and hemodialysis subjects (H-W = 0.94) for men.
In women, the distribution of PON1 allelic frequencies showed Q allele frequencies of 83.72 ± 3.98%, 81.63 ± 3.91%, and 75 ± 6.24%, and R allele frequencies of 16.28 ± 3.98%, 18.37 ± 3.91%, 25 ± 6.24% respectively in healthy, diabetic, and hemodialysis subjects, respectively (Table 3). Our results followed Hardy-Weinberg equilibrium for healthy (H-W = 0.93), diabetic (H-W = 1.03) and hemodialysis subjects (H-W = 0.81) for women.

In men, the crude OR comparing the PON1 R allele with the Q allele was OR1 = 1.02 (95% CI: 0.39-2.64) in diabetic subjects and OR2 = 2.21 (95% CI: 0.95-5.13) in hemodialysis subjects. In women the crude OR comparing the PON1 R allele with the Q allele was OR1 = 1.16 (95% CI: 0.56-2.41) in diabetic subjects and OR2= 1.71 (95% CI: 0.85-3.44) in hemodialysis subjects (Table 3).

We also investigated the allelic frequency distribution of PON1 according to age. Among subjects aged 40-60 years, the frequencies of the PON1 Q allelic were 85.71 ± 3.30%, 87.74 ± 3.18%, and 74.44 ± 4.60%, whereas the frequencies of the R allele were 14.29 ± 3.30%, 12.26 ± 3.18%, 25.56 ± 4.60%, in healthy, diabetic, and hemodialysis subjects, respectively (Table 4). Our results followed Hardy-Weinberg equilibrium for healthy (H-W = 0.94), diabetic (H-W = 1.01) and hemodialysis subjects (H-W = 0.84) for subjects aged 40-60 years. In subject aged

40-60 years, the crude OR comparing the PON1 R allele with the Q allele was OR1 =0.84 (95% CI: 0.37-1.90) for diabetic subjects and OR2 = 2.06 (95% CI: 1-4.22) for hemodialysis subjects.
Among subject aged 60-80 years, the frequencies of the PON1 Q allele were 83.33 ± 4.81%, 81.82 ± 4.75%, and 85.22 ± 3.78%, whereas the frequencies of the PON1 R allele were 16.67 ± 4.81%, 18.18 ± 4.75%, and 14.77 ± 3.78% in healthy, diabetic, and hemodialysis subjects, respectively (Table 4). Our results followed Hardy-Weinberg equilibrium for healthy (H-W = 0.90), diabetic (H-W = 0.90) and hemodialysis subjects (H-W = 0.97) for subjects aged 60-80 years.

In subjects aged 60-80 years, the crude OR comparing the PON1 R allele with the Q allele was OR1 = 1.11 (95% CI: 0.53-2.31) for diabetic subjects and OR2=0.87 (95% CI: 0.4-1.86) for hemodialysis subjects.

Table 4 : Paraoxonase 1 allelic frequency distribution as a function of age in healthy, diabetic and hemodialysis subjects

	Allelic frequency	Healthy subjects	Diabetic subjects	Hemodialysis subjects	OR1 (95%IC)	OR2 (95%IC)
[40-60 years]	Q	85.71 ± 3.30%	87.74 ± 3.18%	74.44 ± 4.60%	1	1
	R	14.29 ± 3.30%	12.26 ± 3.18%	25.56 ± 4.60%	0.84 (0.37-1.90)	2.06 (1-4.22)
[60-80 years]	Q	83.33 ± 4.81%	81.82 ± 4.75%	85.22 ± 3.78%	1	1
	R	16.67 ± 4.81%	18.18 ± 4.75%	14.77 ± 3.78%	1.11 (0.53-2.31)	0.87 (0.4-1.86)

The distribution of allele frequencies of each polymorphism was compared between case and control subjects using χ^2 test. OR1 was calculated for diabetic versus healthy subjects, whereas OR2 was calculated for hemodialysis versus healthy subject

Table 5: Crude odds ratios according to PON1 phenotype distribution in diabetic and hemodialysis subjects

PON1	OR1 (95%IC)	OR2 (95%IC)
Q	1	1
QR	1.37 (0.62-3.04)	1.52 (0.69-3.35)
RR	0.84 (0.22-3.25)	1.97 (0.63-6.21)

OR1 was calculated for diabetic versus healthy subjects, whereas OR2 was calculated for hemodialysis versus healthy subjects.

We also investigated the crude OR associated with the PON1 QQ, QR and RR phenotypes according to age in diabetic and hemodialysis subjects. Among diabetic subjects aged 40-60 years, the crude OR comparing the QQ phenotype with the QR phenotype was OR1=1.15 (95% CI: 0.44-3.00), whereas the OR comparing the QQ phenotype with the RR phenotype was OR1 = 0.35 (95% CI: 0.03-3.50). Among diabetic subjects aged 60-80 years,

the crude OR comparing the QQ phenotype with QR phenotype was OR1 = 2.08 (95% CI: 0.47-9.29), whereas the OR comparing QQ phenotype with the RR phenotype was OR2=1.56 (95% CI: 0.24-10.19).

Among hemodialysis subjects aged 40-60 years, the crude OR comparing the PON1 QQ phenotype with the QR phenotype was OR2=1.33 (95% CI: 0.48-3.69), whereas the crude OR comparing the QQ phenotype with the RR phenotype was OR2= 3.46 (95% CI: 0.83-14.49) for RR. Among hemodialysis subjects aged 60-80 years, the crude OR comparing the PON1 QQ phenotype with the QR phenotype was OR2 = 2.27 (95% CI: 0.56-9.27), whereas the crude OR comparing QQ phenotype with the RR phenotype was OR2= 0.76 (95% CI: 0.10-5.76) (Table 6).

Table 6 : Crude odds ratio in diabetic and hemodialysis subjects and the associated risk of PON1 Q192R polymorphism

	PON1	OR1 (95%IC)	OR2 (95%IC)
[40-60 years]	Q	1	1
	QR	1.15 (0.44-3.00)	1.33 (0.48-3.69)
	RR	0.35 (0.03-3.5)	3.46 (0.83-14.49)
[60-80 years]	Q	1	1
	QR	2.08 (0.47-9.29)	2.27 (0.56-9.27)
	RR	1.56 (0.24-10.19)	0.76 (0.10-5.76)

OR represents the adjusted odds ratio. OR1 was calculated for diabetic versus healthy subjects, whereas OR2 was calculated for hemodialysis versus healthy subjects.

Discussion:-

Many studies support the role of oxidative stress in the pathogenesis of diabetes and chronic renal failure [16-19]. The main findings of this study were that the frequency of the PON1 192R phenotype tended to be higher in diabetic and hemodialysis subjects than in healthy controls. Factors affecting serum PON1 activity include type 1 and 2 diabetes mellitus, chronic renal disease, familial hypercholesterolemia, and inflammatory disorder such as rheumatoid arthritis and hemodialysis [19-26].

In our study, we observed an increase of Vit.E/TC ratio in PON1 QQ phenotype by comparison to RR phenotype, which may suggest reduced lipid peroxidation and lower oxidative stress in subject with PON1 QQ phenotype. It is known that the PON1 QQ phenotype inhibits the initiation and propagation of lipid peroxidation [23-25]. Vitamin E is a chain breaking antioxidant stopping the propagation of lipid peroxidation [27]. It also suggested that PON1 polymorphism may influence the ability of vitamin E to impede the development of atherosclerosis and to prevent inflammation.

We observed an increase of Vit E /CT ratio in PON1 QQ carriers by comparison to PON1 RR carriers in diabetics and hemodialysis indicating changes in lipid profile in these subjects. Aviram et al. reported that, PON1 inhibits efficiently the production of lipid peroxides at 61% and aldehyde compounds at 58% [26]. PON1 also inhibits LDL oxidation efficiently if it presents in initiation of lipid peroxidation step. But the PON1 RR variant inhibits production of lipid peroxidation at 46% and aldehyde compounds in 38% [26].

Published data on the association between PON1 polymorphisms and coronary heart disease have yielded controversial results [28]. Study from Bub et al. indicates that, PON 1 Q192R polymorphism is associated with reduced lipid peroxidation in R-allele-carriers but not in QQ homozygous elderly subjects on a tomato-rich diet [28].

The relationship between the two human PON1 amino acid variants, the Leu55Met and the Gln192Arg polymorphism, and the risk of cardiovascular disease is also documented in this study. The main purpose is to investigate the link between the PON1 allele frequency distribution, crude odds ratio and the risk of cardiovascular diseases development in a Moroccan population.

Our result showed a trend toward increased risk of cardiovascular disease in QR carriers vs QQ carriers in diabetic (OR1＝ 1.37; 95% CI: 0.62-3.04) and hemodialysis subjects (OR2=1.52; 95% CI: 0.69-3.35).

The observed differences between phenotypic groups may be related to the Gln192Arg polymorphism of PON1; however, no statistically significant association with cardiovascular disease risk was demonstrated in the present study. The Gln192Arg polymorphism of PON1 indicates changes from glutamine (the Q variant) to arginine (the R variant) at position 192 of the amino acid sequence [8,9]. These changes may affect its hydrolytic activity [26]. Arginine is a conditionally essential amino acid serving as a substrate for protein synthesis, L-arginine is the pre-cursor for nitric oxide (NO), is responsible for free radical production [[9, 29]. These results are in line with other studies that investigated PON1 polymorphism related to cardiovascular diseases risk [22, 30, 31].

We also observed that, the PON1 192Q allele frequency (86.62%; 85.46% and 79.78%) is higher than that of PON1 192R allele frequency (13.37%; 14.53% and 20.22%) respectively in healthy, diabetic and hemodialysis subjects. Van Den Berg et al. observes comparable R-allele frequencies for subjects with normal Glucose Tolerance 32% and Newly diagnosed Type 2 diabetes 25% [32]. Although the odds ratios tended to be higher among R-carriers, the confidence intervals included unity, indicating that these associations did not reach statistical significance. Therefore, the odds ratios tended to be higher among R-carriers, the confidence interval included unity, indicating that these associations did not reach statistical significance. Therefore, these findings should be interpreted as suggestive rather than conclusive.

In the present study, we have shown that, PON1 192R phenotype may be associated with less favorable oxidative stress in diabetic and hemodialysis patients. As a function of age, we note a tendency toward increased risk of cardiovascular diseases in hemodialysis (OR2 = 2.06; 95% CI: 1-4.22) and diabetic subjects (OR1=1.11; 95% CI: 0.53-2.21) when comparing allelic frequencies homozygosity for QQ variant and RR variant. These results may suggest increased oxidative stress in diabetic and hemodialysis subjects.

Paraoxonase 1 Arg 192 Gln allelic frequency distribution showed a high frequency of the R allele in female diabetic. Our diabetic women were obese and hypertensive. Hypertension affects the PON1 activity and increases the susceptibility to atherosclerosis [33]. It also compromises the capacity of their HDL to prevent the accumulation of lipid peroxide on human LDL [34]. Lawlor et al. study indicated that, there was a high risk of cardiovascular diseases among the findings from the British Women's Heart and Health cohort study and a meta-analysis over 60 years [35]. PON1 allelic frequency distribution is elevated in male and female hemodialysis patients because of dialysis process. Dialysis process produces free radicals responsible for oxidative damage in these subjects [36, 37]. Hemodialysis alters also lipid profile, total antioxidant capacity, vitamin A, E and C concentration in humans and also increase cardiovascular diseases development [38]. To our knowledge, few studies provide data of the PON1 Q192R polymorphism related to cardiovascular diseases in hemodialysis patients [39]. The low frequency of the RR phenotype reduced statistical precision and likely contributed to the wide confidence intervals observed in several analyses.

Our study has shown that, diabetic subjects and hemodialysis patients carrying RR and QR had high triglycerides level when compared to healthy control carrying QQ. These results are in line with other studies [40, 41]. Jarvik et al. reported that PON1 is a better predictor of vascular diseases than be the PON1-192 or PON1-55 [40]. This study showed that the R allele alters the reactivity of the paraoxonase [40]. Our result which agrees with other studies suggested that PON1 activity can be used as a cardiovascular disease prediction marker in healthy, diabetic and hemodialysis patients [40, 41]. Our findings suggest that PON1 polymorphisms may influence the lipid profile, particularly it showed that the R allelic frequency of PON1 disturbs serum triglycerides distribution in lipoproteins. Compared with hemodialysis patients, diabetic subjects had better lipid profile (lower triglycerides) because they reported a higher use of lipid medication. Deakin et al. reported that Statins modulates the expression of the PON1 gene and increase serum Paraoxonase [42]. A logical explanation appears to be the higher frequency of the R allele in our hemodialysis patients. These findings may suggest reduced protection against oxidation and a possible increase in cardiovascular risk, although the observed odds ratios were not statistically significant [42, 43]. A

plausible explanation can be the high level of triglycerides in hemodialysis subjects which alter the composition of HDL, VLDL, LDL cholesterol, and finally influence the PON1 binding and its activity [43]. Although higher MDA concentrations were observed in some phenotype groups, several comparisons did not reach statistical significance. Therefore, these findings should be interpreted as indicative of possible biological trends rather than definitive evidence of increased oxidative stress.

A possible contributing factor to the relatively low prevalence of cardiovascular diseasse observed in Morocco may be the traditional Mediterranean dietary pattern, which is rich in fruits, vegetables, olive oil, and other antioxidant-rich foods. Adherence to this dietary pattern has been associated with a reduced risk of cardiovascular disease and premature mortality [44].

In conclusion, diabetic and hemodialysis patients exhibited reduced paraoxonase activity and increased oxidative stress markers compared with healthy controls. Although a higher proportion of R-phenotype carriers was observed among hemodialysis patients, the estimated odds ratios did not demonstrate statistically significant associations with cardiovascular disease risk. These findings should therefore be considered exploratory and require confirmation in larger prospective studies using direct molecular genotyping.

Study limitations: Several limitations should be acknowledged. First, PON1 Q192R status was inferred using a phenotypic enzymatic method rather than direct molecular genotyping. Second, the number of subjects classified as RR phenotype was relatively small, resulting in wide confidence intervals and limited statistical power for subgroup analyses. Third, the cross-sectional design precludes any causal inference between PON1 phenotype distribution and cardiovascular disease risk. Fourth, residual confounding is likely because important variables such as statin use, hypertension, obesity, duration of diabetes, duration of hemodialysis, and concomitant medications were not fully controlled in the present analyses. Finally, cardiovascular outcomes were not prospectively assessed.

In addition, stratified analyses according to age and sex should be interpreted with caution because the number of subjects within some phenotype subgroups, particularly RR carriers, was small, thereby reducing statistical power and increasing uncertainty around the estimated odds ratios.

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