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ORIGINAL ARTICLE

Q192r Paraoxonase 1 Gene Polymorphism among Rheumatoid Arthritis Patients in Zagazig University Hospitals

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ABSTRACT

Background: Rheumatoid arthritis patients (RA) have expanded cardiovascular morbidness and death rate. Paraoxonase1 (PON1) polymorphism has been related to expanded cardiovascular hazard. Aim of the work was to assess the relationship between PON1 gene polymorphism and RA. **Methods:** A case control study was conducted in Rheumatology and Rehabilitation Department in cooperation with Medical Biochemistry and Biology Department, Zagazig University Hospitals on 106 subjects. They were divided randomly into case and control groups, full history was taken from all patients, also locomotor examination, laboratory and radiological evaluation were done. Genotyping of PON1 gene polymorphism was studied in both groups **Results:** Regarding PON1 polymorphism genotypes, the difference in PON1 genotypes frequency was not significant among RA patients and controls (P>0.05). Levels of total triglycerides, HDL and LDL were different significantly in both groups. **Conclusion:** Although RA patients had higher cardiovascular risk, PON1 Q192R polymorphism was not prominent in RA patients contrasted with controls.

Key words: Rheumatoid arthritis, PON1 polymorphism, cardiovascular risk.

INTRODUCTION

Rheumatoid arthritis (RA) is a long term, inflammatory, immune system disease that not only influences the joints but also produces autoantibodies targeting adjusted self-epitopes. **(1)** RA is a chronic, immune-mediated inflammatory disease with evidence of general autoimmunity.**(2)**

Patients with RA have significantly higher incidence of cardiovascular diseases and death rate. Atypical function of high density lipoprotein (HDL) was suggested as a possible mechanism for this expanded cardiovascular hazard.**(3)** HDL promotes cholesterol efflux and prevents low-density lipoprotein (LDL) oxidation as products resulting from LDL oxidation are pro inflammatory.**(4)**

Paraoxonase1(PON1) activity varies to a large extent in populations and this difference relies on genetic polymorphisms mostly the Q192R polymorphism which is related with expanded cardiovascular hazard.**(4)** Thus, the aim of current work was to assess the relationship between PON1 gene polymorphism and increased incidence of RA and its relation to dyslipidemia in these patients.

METHODS

Study design and subjects

This case control study was conducted in Rheumatology and Rehabilitation Department in cooperation with Medical Biochemistry and Biology Department, Zagazig University Hospitals.Written informed consent was obtained from all participants and the study

was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans. The sample calculated to be 106 divided into 2 groups: 53 RA patients (Group 1), 53 age and sex matched apparently healthy volunteers (group 2).Inclusion criteria included: Age > 16 years old, all patients who fulfill at least six points of the revised 2010 ACR/EULAR classification criteria for Rheumatoid Arthritis.[5]Exclusion criteria included: Patients having overlap with other rheumatologic disease as systemic sclerosis, systemic lupus erythematosus (SLE), polymyositis, patients with renal diseases, hepatic diseases, hypertension or coronary heart disease dating before the onset of RA and smokers. All patients were subjected to full history taking, and thorough clinical examinations. C-reactive protein (CRP)[6], erythrocyte sedimentation rate (ESR)[7] and lipid profiles were assessed. Also, serum rheumatoid factor (RF) [8] and anti cyclic citrullinated peptide (anti-CCP) [9] were measured. Number of swollen joint (NSJ), number of tender joints (NTJ), patient's global assessment visual analog scale (VAS), disease activity score in 28 joints (DAS28) were all used to assess disease activity [10]. The modified Health Assessment Questionnaire disability index (MHAQ)[11] was used to evaluate disease disability. X-rays of the hands were obtained in all patients.

Determination of 192 PON1 gene polymorphism

Blood DNA extraction was done using standard procedures. PON1 192 gene polymorphism was conducted by restriction fragment length polymorphism- polymerase chain reaction (RFLP-PCR).[12] The primers 5`-TATTGTTGCTGTGGGACCTGAG-3`(forward) and 5`CACGCTAAACCCAAATACATCTC-3`(reveres) were used to obtain the 99-bp DNA fragment compassing the PON1 192 polymorphism. The amplified products (Q allele at 99-bp fragment, and R allele at 65-bp and 34-bp fragments) were digested with *Alw*I, and were analyzed by electrophoresis on a 4% agarose gel stained with 0.1% ethidium bromide. The digested fragments were visualized on a UV transilluminator.

Statistical analysis

The collected information were coded, entered, presented, and analyzed by computer via a data base software program, Statistical Package for Social Science (SPSS) version 12.0.1 (SPSS, Inc., Chicago, IL, USA). Parametric variables were represented as the mean and standard deviation (SD), and non parametric data expressed as median and range. Chi square (X^2) or Fisher tests were used to detect relation between different qualitative variables. For quantitative variables, t-test (t) and one-way ANOVA (F) with post hoc were used for normally distributed data, while Mann Whitney U test used for comparison of median of two independent samples if two variables are not normally distributed. P value≤0.05 means statistically significant.

RESULTS

Demographic, clinical and laboratory characteristics of RA patients& controls:

Table (1) showed non-significant difference between both groups (patients and controls) as regards to age and sex. The range of disease duration was from 1 to 30 years. Also, body mass index among patients and controls had highly significant difference (P<0.001).Table (2) showed that a highly significant difference between studied groups as regards ESR and CRP (P<0.001). Concerning lipid profile, it differed significantly between RA patients in relation to controls regarding TG, HDL and LDL levels (P<0.001).

Genotyping of RA patients & controls:

Table (3) showed statistically insignificant difference in genotyping of patients and control (P>0.05). Carriers of genotype QQ and genotype QR were 2.29 and 1.46 times more likely to develop RA (OR=2.29, OR=1.46 respectively). As regards to the allelic frequencies of the PON1 polymorphism of RA patients and controls, figure (1) showed that the

frequencies of allele Q & R were insignificant in RA patients in relation to controls (P>0.05). Table (4) showed there was statistically insignificant relation between genotyping and disease activity measures and functional status in RA patients (P>0.05).

Table 1. Demographic data among studied groups:

Items	*Studied groups*		*Test*	*P*
	Group I (patients) (no=53)	***Group II (control) (no=53)***		
Age per years *Mean ±SD*	*43.6±10.6*	*41.2±9.7*	*t=1.2*	*0.23(NS)*
Gender ***Females*** *no (%)* ***Males*** *no (%)*	*49 (92.5)* *4 (7.5)*	*49 (92.5)* *4 (7.5)*	X_2*=1.7*	*0.58(NS)*
BMI(kg/m2) *Mean ±SD*	*28.9±4.7*	*24.3±1.5*	*t=6.8*	***0.00001(HS)***
Family history *no (%)*	*3(5.7)*	*0*		*0.24*(NS)*

*= fisher exact test, t =t test of significant, χ^2 = Chi square test, NS= Non Significant, HS= highly significant,S = Significant, SD= Standard Deviation, BMI= Body mass index.

Table 2. Laboratory variables among studied groups:

	Group I (patients) (no=53)	*Group II (control) (no=53)*	*Test*	*P*
ESR (mm/hr) *median (Range)*	*35(5-90)*	*7(5-26)*	*MW*	***0.00001***
CRP (mg/L) *median (Range)*	*7(0.4-96)*	*2.4(0.4-5.5)*	*MW*	***0.00001***
Hemoglobin (g/dl) *mean ±SD*	*11.9±1.3*	*11.5±1.6*	*t=1.7*	*0.08*
WBCs (10^3/ml) *mean ±SD*	*6.9±2*	*7±1.8*	*t=0.006*	*0.99*
Platelet (10^3/ml) *median (Range)*	*241(115-800)*	*227(155-396)*	*MW*	*0.3*
AST (U/L) *median (Range)*	*19(8.1-60.70)*	*19(8.60-32)*	*MW*	*0.47*
ALT (U/L) *median (Range)*	*14.9(6.3-83.6)*	*14(6.3-31)*	*MW*	*0.33*
Albumin (g/dl) *mean ±SD*	*4±0.5*	*4.1±0.4*	*t=0.8*	*0.41*
Creatinine (mg/dl) *mean ±SD*	*0.6±0.2*	*0.7±0.1*	*t=0.9*	*0.37*
BUN (mg/dl) *median (Range)*	*12.2(1.8-36)*	*9.2(6-18.9)*	*MW*	*0.13*
Cholesterol (mg/dl) *mean ±SD*	*184.2±43.5*	*172.2±21.9*	*t=1.8*	*0.07*
TG (mg/dl) *median (Range)*	*135.1(55.7-270)*	*88(50.2-160.2)*	*MW*	***0.0001***
HDL (mg/dl) *mean ±SD*	*59.4±12.8*	*77.7±16*	*t=6.5*	***0.0001***
LDL (mg/dl) *mean ±SD*	*108.2±30.2*	*65.6±22.4*	*t=8.2*	***0.0001***

MW= Mann-Whitnney test of sig, t =t test of significant ,WBCs: white blood cells, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, ALT: Alanine transaminase, AST: aspartate transaminase, BUN: blood urea nitrogen**,** TG:triglycerides, HDL= High density lipoprotein, LDH=Low density lipoprotein, .RF: rheumatoid factor, AntiCCP: anti cyclic citrullinated peptide.

Table 3. Genotypes frequency of PON1 Q192R polymorphism in RA patients &controls:

	Group I (patients) (no=53)	*Group II (control) (no=53)*	*OR (95%CI)*	*Test*	*P*
***QQ** no(%)*	*28(52.8)*	*35(66)*	*2.29*	*χ 2=2.4*	*0.3(NS)*
***QR** no(%)*	*14(26.4)*	*12(22.6)*	*1.46*		
***RR** no(%)*	*11(20.8)*	*6(11.3)*	*1*		

χ^2 = Chi square test, NS: Non Significant, OR: odds ratio.

Table 4. PON1 genotypes frequency in relation to disease activity measures and functional status.

	Genotyping			*Test*	*P*
	QQ(no=28)	***QR(no=14)***	***RR(no=11)***		
NTJ					
Mean ±SD *median(Range)*	*12.4±10* *10(0-28)*	*12±10* *8(1-28)*	*12.5±11.7* *5(0-28)*	*KW*	*0.96*
NSJ					
Mean ±SD *median(Range)*	*3±4* *2(0-20)*	*3.±4* *2(0-13)*	*1.45±3* *0(0-10)*	*KW*	*0.11*
DAS28					
Mean ±SD *median(Range)*	*5.2±1.6* *5.6(2.02-8.03)*	*5±1.5* *5.1(2.09-6.9)*	*4.6±1.6* *4.5(2.7-7.05)*	*KW*	*0.65*
MHAQ					
Mean ±SD *median(Range)*	*0.88±0.64* *0.94(0-2.14)*	*2.2±5.5* *0.75(0-21.13)*	*0.83±0.6* *0.63(0.13-1.87)*	*KW*	*0.99*
RF (U/ml)					
median (Range)	*135.5(2.2-480)*	*62(3.3-364.4)*	*40.3(7.8-528)*	*KW*	*0.39*
AntiCCP (U/ml)					
median(Range)	*183(2.2-219)*	*219.4(.88-303.2)*	*36.2(7-296.8)*	*KW*	*0.23*

KW=Kruskall Wallius test, S: significant, SD: Standard Deviation , NTJ: number tender joint, NSJ: number swollen joint, DAS: disease activity score, MHAQ: modified health assessment questionnaire.

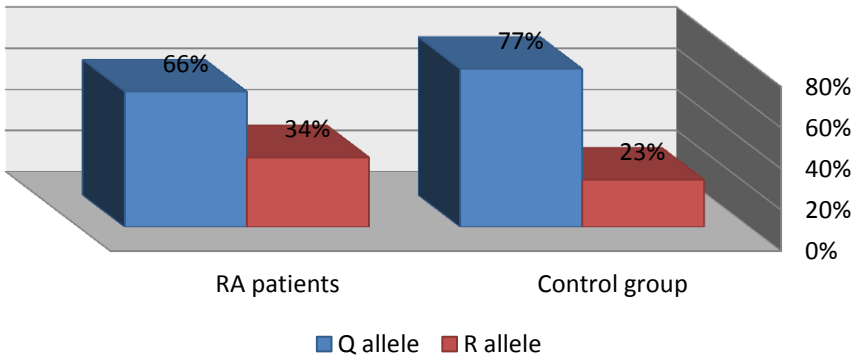

Figure 1. Percent Alleles of PON1 Q192R polymorphism in RA patients and controls.

DISCUSSION

Patients with RA have significantly expanded cardiovascular morbidness and mortality. Atypical function of HDL was suggested as a possible mechanism for this expanded cardiovascular hazard.**(3)** PON1 is an enzyme related to HDL which promotes anti-inflammatory and antioxidant properties of HDL by blocking formation of oxidized LDL and deactivate oxidized phospholipids. Serum PON1 has been found to be directly involved in the pathogenesis of atherosclerosis.**(13)**

The current study included 53 patients suffering from RA; four of them were males and 49 were females. The control group was normal volunteers apparently free from any disease which included 49 females and four males. Regarding demographic and clinical characteristics, the mean age of patients and control groups was 43.6±10.6 and 41.2±9.7 respectively. There was a difference of significance between the studied groups in BMI ($P<0.001$). The duration of the disease in patients group ranged between 1-30 years with mean of 8.5±6.2 and there was three patients only with family history of RA.

Similar to other studies in Egyptian populations**, Elfasakhany et al.** found that the majority of the patient and control groups were females with no statistically difference between both groups Regarding age and BMI.**(14)** In matching with the work conducted by **El Hewala et al.** they demonstrated that RA patients and the volunteers differed significantly as regards RF, ESR and CRP.**(15)**

Regarding lipid profile, there was highly statistical difference between patients and control groups in TG,HDL and LDL. The above mentioned findings were similar to those of **Vottery et al.** who demonstrated that patients with very active disease had higher levels compared to controls.**(16)**In agreement with **Attar**, RA patients in his study demonstrated higher prevalence of elevated total cholesterol and low-density lipoprotein denoting that hyperlipidemia is common among RA patients with increased cardiovascular risk.**(17)**. On the other hand, these current findings were different from those reported by **Tanimoto et al.** who found that there was no statistically significant difference between RA patients and control groups regarding total cholesterol, TG, HDL, and LDL.**(18)**Also, **Elfasakhany et al.** reported that no statistically significant difference was found among the studied groups (RA patients and control) as regards total cholesterol, TG, HDL, and LDL. The difference in lipid profile levels found in multiple studies may be as a result of discrepancy in the populations and in activity of the disease.**(14)**

This study also showed that, there was no significance difference ($P>0.05$) between patients and control groups regarding genotypes frequency of PON1 Q192R polymorphism. Carriers of genotype QQ and genotype QR were 2.29 and 1.46 times more likely to

develop RA. **Elfasakhany et al.** reported the same results regarding genotypes frequency of PON1 Q192R polymorphism, so PON1 Q192R polymorphism in Egyptian population is not associated with higher incidence of RA.**(14)** Also, **Hashemi et al.** suggested that PON1 Q192R polymorphism in Iranian population is not correlated with increased risk for RA.**(19)**

However, the study carried out by **Khoja et al.** which stated that RA and control subjects differed significantly regarding PON1Q192R genotypes. This difference might be as a result of distinction in ethnic populations.**(20)** In RA Japanese patients, **Tanimoto et al.** found that QQ PON1 genotype was apparently fewer in RA patients than healthy subjects while there is no difference in RR genotype frequency between both groups.**(18)**

Regarding Alleles frequency of PON1 Q192R polymorphism among the included groups, it was observed that there was no statistically significance difference (P>0.05) between both of them. This is matching with the study of **Elfasakhany et al.** who found that there was no difference between studied groups as regards alleles frequency of PON1 Q192R polymorphism.**(14)**

PON1 genotypes distribution in relation to ESR and Anti-CCP among RA group, found that there was no statistically significant relation (P>0.05). The current results disagreed with the findings of **Tanhapour et al.** that revealed RA patients who carried Q alleles had higher concentrations of anti-CCP-antibody.(21)

CONCLUSION

The current study concluded that PON1 Q192Rpolymorphism was not associated with increased risk for rheumatoid arthritis despite abnormal HDL and dyslipidemia in RA patients. Future studies are recommended to investigate the PON1 polymorphism in a bigger population which may explain the definite connection among PON1 polymorphism and cardiovascular hazard in RA.

Conflict of Interest: Nothing to declare.
Financial Disclosures: Nothing to declare.

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