ROLE OF GENE POLYMORPHISM OF INTERLUKIN-1 AND ITS RECEPTOR ANTAGONIST IN CAROTID ATHEROSCLEROSIS OF RHEUMATOID ARTHRITIS PATIENTS

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ABSTRACT

Aim: To investigate the possible association between genes polymorphisms of the interlukin 1- β (IL-1 β) and IL-1 receptor antagonist (IL-1 ra) and susceptibility to carotid atherosclerosis in rheumatoid arthritis.

Subjects and methods: The study included two groups. Group I: 120 premenopausal female patients who fulfilled the American College of Rheumatology classification criteria for rheumatoid arthritis (RA) and Group II: 120 premenopausal female's apparently healthy subjects were selected as control group. Cases and controls symptomatic for atherosclerosis or having traditional risk factors for atherosclerosis were excluded. Blood was obtained for DNA determination of IL-1 β gene and IL-1 Ra gene polymorphisms from both cases and controls.They were subjected to carotid ultrasound examination to measure carotid inimal medial thickness (CIMT) in addition to detailed history and physical examination and

Results: It was found that RA patients who had the IL 1-β allele 1 showed an increased risk to develop atherosclerosis with CIMT ≥ 0.9mm (OR = 13.2, P = 0.000) compared with RA patients with IL 1-β allele 2 (OR = 11.3, P = 0.000). While in control there was no risk to develop atherosclerosis with IL1- β alleles. Also, It was found that RA patients who had the IL1-ra allele 1 showed an increased risk to develop atherosclerosis with CIMT ≥ 0.9mm (OR = 39, P = 0.000) compared with RA patients with other alleles of IL1-ra (OR = 14.3, P = 0.000) .While in control there were no risk to develop atherosclerosis with IL1-ra alleles.

Conclusion: These data suggest that allele 1 of the IL-1 β gene and allele 1 of the IL-1ra gene represent a susceptibility factor in the development of carotid atherosclerosis in RA patients.

Keywords: Rheumatoid arthritis, IL-1, IL-1ra, Atherosclerosis, Polymorphism.

1. INTRODUCTION

Rheumatoid arthritis (RA) is a common autoimmune disease in which a combination of risk alleles from different susceptibility genes predisposes the patient to development of clinical symptoms following exposure to as yet unknown environmental factors **(1).** In addition, multi-factorial cardiovascular (CV) disease in RA has been shown to be partially genetically determined; it has also been reported that changes in the lipid profile may occur in individuals up to 10 years before the diagnosis of RA, indicating a potential genetic link between RA and dyslipidaemia **(2).**

Despite the excess risk of vascular disease in patients with RA being of a similar magnitude to that seen in diabetes, patients with RA did not receive additional CV disease screening in primary care, although this was achieved in patients with diabetes **(3).**

An increased Carotid intima-media thickness CIMT is present in patients with rheumatoid arthritis, compared with control individuals and is thought to indicate premature atherosclerosis **(4).**

Interlukin 1 (IL-1) and IL-1 receptor antagonist gene variations are associated with over-expression of inflammatory mediators are also associated with increased risk of cardiovascular events **(5)**. And further study is needed to understand the relation of these polymorphisms to atherosclerosis in RA.

2. SUBJECTS AND METHODS

2.1. Participants:

Subjects divided into 2 groups. Subjects were divided into: Group I: includes (120) premenopausal female patients attending to rheumatology and rehabilitation department of Zagazig university hospitals were included in this study who fulfilled the American College of Rheumatology classification criteria for RA **(6).** Their ages ranged from (26 - 42) years and the duration of the joint disease ranged from (0.9-12) years. Group II: includes (120) premenopausal females. Their ages ranged from (25y - 40y) years, apparently healthy subjects were selected as control group. We exclude patients with hypertension, dyslipidemia (total cholesterol and/or triglyceride levels in fasting plasma >250 mg/dl, and >160 mg/dL),diabetes mellitus (fasting plasma glucose concentration included in this study had to be <110 mg/dL),renal insufficiency

(serum creatinine values had to be <1.4mg/dL), obesity [body mass index (BMI) > 30 kg/m²], evidence CV disease or family history of coronary heart disease or received drugs affecting the CV system antihypertensive or anti-aggregant drugs, nitrates, and statins) were also ruled out, smoking or previous history of smoking 5 years ago and postmenopausal women, neoplasms, or other connective tissue diseases.

All patients were subjected to history taking, and Locomotor examinations were preformed to all patients including tender joint count (TJC) and swollen joint count (STC). Disease activity was determined on the basis of defined parameters [The number of swollen and tender joints, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP)]. X-rays of the hands were obtained in all patients. Disease severity was determined on the basis of defined parameters [rheumatoid factor (RF) and X-ray erosion].

2.2. *Biochemical analysis*:

Blood samples were drawn from all subjects after an overnight fast. The following investigations were measured [ESR, CRP, RF, blood urea nitrogen (BUN), fasting blood sugar,serum creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lipids profile including (triglycerides, total blood cholesterol)].

2.3*. Isolation of DNA:*

Five ml of peripheral venous blood samples were withdrawn from each subject under complete aseptic condition and were divided into 2 portions. One ml collected on potassium EDTA (1mg/ml) for DNA extraction, three ml were left for 30-60 minutes for clotting then centrifuged at 3000 rpm for 10 minutes; serum samples were separated into another set of tubes and kept frozen at -20ºC till the time for assay. All the work was carried out in the Biochemistry Department. All the reagents were highly purified analytical PCR-(polymerase chain reaction) materials. All the tubes, tips pipettes used for DNA extraction were DNAse, RNAse free tubes.

2.4. *Amplification of the IL-1 β gene polymorphism:* **(7)**

The polymorphism was amplified with following forward primers: 5' GTTGTCATCAGACTTTGACC3; and reverse primer 5'TTCAGTTCATATGGACCAGA3. PCR Reaction was performed in a final volume of 50 µl reaction mixture containing 8 µl of the genomic DNA samples, 2 µl of each primer, 30 µl 2x Super Hot PCR Master Mix (from Bioron Gmb H) and 10 µl DdH2O. PCR protocol: Amplification was performed using a thermal cycler Perkin-Elmer 9600 (Perkin-Elmer, Norwalk, CT).Three cycle of 90 s at 97 ºC, 90 s at 55 ºC (annealing), and 60 s at 74 ºC (extension). 32 cycles of 30 s at 97 ºC, 30 s at 55°C, and 30 s at 74°C.One cycle for 10 min at 72°C. TaqI digestion of the 249-bp fragments resulted in fragments that either remained intact (allele 2) or were cut in two fragments of 135 bp and 114 bp, respectively (allele 1).Fragments were analysed by electrophoresis on 3% agarose gels containing 0.1% ethidium bromide.

2.5. *Amplification of IL-1 ra gene polymorphism:* **(7)**

The polymorphism was amplified with following forward primers 5' CTCAGCAACACTCCTAT3'; and reverse primer 5' TCCTGGTCTGCAGGTAA 3'. PCR Reaction was performed in a final volume of 50 µl reaction mixture containing 8 µl of the genomic DNA samples, 2 µl of each primer, 30 µl 2x Super Hot PCR Master Mix and and 10 µl DdH2O. PCR protocol: Amplification was performed using a thermal cycler Perkin-Elmer 9600 (Perkin-Elmer, Norwalk, CT).One cycle for 1 min of initial denaturation at 94 ºC.35 cycles of 1 min of denaturation at 94 ºC, 1 min of annealing at 60°C, and 1min of elongation at 72°C. One cycle for 10 min of final elongation at 72°C.The PCR products of 410 bp (allele I = four repeats of the 86-bp region), 240 bp (allele 2 = two repeats), 500 bp (allele 3 = five repeats), 325 bp (allele 4 = three repeats) and 595 bp (allele 5 = six repeats) were analysed by electrophoresis on a 2% agarose gel stained with 0.1% ethidium bromide.

2.6. *Ultrasound image analysis*:

Ultrasonographic evaluation of the common carotid artery (CCA) intima-media thickness (IMT) was performed as an early marker of generalized atherosclerosis and vascular risk. A Toshiba (diagnostic ultrasound system), model (SSA 270 A), B-mode with pulsed Doppler flow imaging system, using a 6 MHz linear array transducer. The patients were investigated in the supine position with the head slightly turned from the sonographer. The carotid arteries are examined bilaterally in the areas of the common carotid artery far wall (1 cm proximal to the dilatation of the carotid bulb), the carotid bifurcation (1 cm proximal to the flow divider), and the internal carotid artery (1 cm distal to the flow divider) on the left and right sides and calculation of the mean of the six readings. The following grading after calculation of the mean of IMT, IMT normal if < 0.9 mm, while values ≥ 0.9mm were considered to be indicative of thickened intima**.** If the maximum IMT, which is the highest IMT value among the six segments

studied > 1.3mm an indicative of atherosclerotic plaque **(8).**

2.7. *Statistical analysis:*

Analyses were performed using SPSS version 12.0.1 (SPSS, Inc., Chicago, IL, USA). Parametric data (normally distributed) were represented as mean and standard deviation (SD), while non-parametric data were represented as median and range. For qualitative data, Chi-Square (X2) tests and fisher exact were used to test of association between a factor and an outcome. For quantitative data the test used to compare between two groups was t test (parametric, unpaired), the Mann-Whitney U test (non parametric, unpaired data). Correlation coefficient was done to study the relation between variables. A P value <0.05 was considered statistically significant, and P value < 0.001 was considered highly significant. For risk assessment Odd̊ s ratio and confidence interval (95%CI) were also calculated to determine the best model for prediction.

3. RESULTS

3.1. *Characteristic data of the two studied groups (Table 1):* This table showed that group I consisted of 120 females, their ages ranged from 26 to 42 years with a mean±SD of 34.4 ± 4.1. While group II consisted of 120 females, their ages ranged from 25 to 40 years with a mean±SD of 33.4 ± 4.5. As regards the BMI, in group I it ranged from 25-29.9 with a mean +SD of 27.9±1.7, while in group II it ranged from 22.3-29 with a mean +SD of 25.9±1.8. The mean of the systolic blood pressure in group I was 118.0±9.2, and in group II it was 116.5 ±6.1.While the mean of the diastolic blood pressure was 78.7±6.8in group I, and it was 77.2±5.9 in group II. As regards all the above parameters, we found non-significant difference between both studied groups (P > 0.05).

When we studied laboratory variables of both groups , it was observed that there was a highly significant difference between both of them as regard RF (P < 0.001),and there were significant difference between both groups as regard serum levels of ESR and CRP (P < 0.05). While there were non-significant difference between them as regard serum lipid profiles, serum creatinine, blood urea nitrogen, AST and ALT (P > 0.05).

3.2. *Sonographic features of the studied groups (Table 2):*

As regards the IMT of the carotid artery, the mean ±SD of IMT was 0.89 ±0.2 in group I, and 0.70±0.09 in group II. There was a highly significant increase in the intima-media thickness of the carotid arteries in group I more than group II (P < 0.001). Also this table showed that there was a highly significant difference in the frequency of CIMT ≥ 0.9 mm in group I than group II (P < 0.001).There were as follow: in group I 67 patients had IMT ≥ 0.9 mm (55.8%), while in group II 6 persons only (5%). Presence of CIMT ≥ 0.9 mm in healthy volunteers may be explained by that 3 of them were pre obese, where their BMI were 29, while 2 persons had a family history of diabetes and one person was ex-smoker. Also this table showed that there was a highly significant difference in the frequency of plaques occurrence in group I than
group II (P < 0.001), where 24 patients in group I had plaques (20%), while in group II 2 persons only had plaque (1.67%).

Table 1: Characteristic data of the two studied groups:

Groups / Variables	Group I no=120	Group II no=120	t/ Mann-whitney	p
Age (years)				
mean± SD	34.4 ± 4.1	33.4 ± 4.5	1.8	0.06
range	26 - 42	25-40		
BMI (kg/m2):				
mean± SD	(27.9±1.7)	(25.9±1.8)	1.22	0.23
range	(25-29.9)	(22.3-29.0)		
Systolic blood pressure (mmHg)				
mean± SD	118.0±9.2	116.5 ±6.1	1.4	0.16
range	(100-140)	(110-130)		
Diastolic blood pressure (mmHg)				
mean± SD	78.7±6.8	77.2±5.9	1.97	0.06
range	(70-90)	(60-90)		
-ESR (mm/h):				
median:	53	30	2.03	0.04*
range:	10-122	15-64		

-CRP (mg/ l):				
median:	4.65	4.9	2.34	0.01*
range:	2.1-20	4-10		
-Rheumatoid factor (u/ml):				
median:	120	14	6.9	0.000*
range:	(5.1- 650)	(5-60)		*
-Lipid profiles (mg/dl):				
Total Cholesterol				
mean± SD	145.9 ± 16.7	141.7 ± 20.2	1.73	0.84
Triglycerides				
mean± SD	76.3 ± 22.7	71.7 ±14.3	1.86	0.06
-Fasting blood sugar (mg/dl):				
mean ± SD	88.3 ± 10.2	87.2 ± 5.9	1.17	0.24
-Kidney function (mg/dl):				
Serum creatinine:				
mean± SD	0.65 ±0.16	0.68 ± 0.12	1.45	0.15
BUN :				
mean± SD	21.4 ± 6.6	22.4 ± 4.8	1.36	0.17
-Liver function (U/L):				
ALT				
mean ± SD	19.3 ± 9.4	20.5 ± 4.8	1.21	0.22
AST				
mean ± SD	20.3 ± 5.7	23.6 ± 5.1	1.8	0.06

*P < 0.05, significant ** P < 0.001, highly significant ESR: Erythrocytes sedimentation rate CRP: C-reactive protein BUN: Blood urea nitrogen. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase.

Table 2: Sonographic features of the studied groups:

Group / Variables	Group I no=120	Group II no=120	t/ X^2	P
CIMT : mean ± SD	0.89± 0.2	0.70± 0.09	8.4	0.000**
CIMT < 0.9 mm no (%):	53 (44.2)	114 (95)	73.2	0.000**
CIMT ≥ 0.9 mm no (%):	67 (55.8)	6 (5)	73.2	0.000**
Plaques no (%)	24 (20)	2 (1.67)	20.88	0.000**

* P < 0.05, significant ** P < 0.001, highly significant CIMT: Carotid intima media thickness.

3.3. *IL1- β genotype and alleles distribution between studied groups:* Table (3) showed that the frequencies of gene 1 and gene 1, 2 of IL1- β were significantly increased in RA patients compared to control group (gene 1 was 42.5% versus 35.0% and gene 1,2 was 31.7% versus 20.0% respectively). Carriers of genotype 1 and genotype 1, 2 were significantly more likely to develop RA [OR = 2.12, CI = (1.11-4.04), P = 0.01 and OR = 2.76, CI = (1.33- 5.75), P = 0.002 respectively]. As regards to the allelic frequencies of the IL1- β polymorphism of RA patients and controls, it was observed that the frequencies of allele 1 genotype were significantly increased in patients with RA compared to control (58.8 versus 45.8) (P < 0.05).Carriers of allele 1 were significantly more likely to develop RA (OR = 1.68 , CI = (1.15 – 2.46), P < 0.05).

3.4. *IL1-ra genotype distribution between studied groups:* Table (4) showed that in RA patients, the frequencies of allele 1, 3 of IL1-ra were significantly increased compared to control group (allele 1 was 30.0% versus 20.0%, where allele 3 was 41% versus 28.8% respectively). Carriers of allele 1 and 3 were significantly more likely to develop RA (OR = 6.67, CI = 2.79-16.3, P = 0.000 and OR = 6.4, CI = 2.78–15.3, P = 0.000 respectively).

Table 3: IL1- β genotype and alleles distribution between studied groups:

Groups / Genotype	Group I no= 120	Group II no=120	OR (95%CI)	P
Gene 2 no (%)	31 (25.8)	54 (45)	1.0	-
Gene 1 no (%)	51 (42.5)	42 (35)	2.12 (1.11-4.04)	0.01*
Gene 1, 2 no (%)	38 (31.7)	24 (20)	2.76 (1.33- 5.75)	0.002*
Alleles	**Group I no= 240**	**Group II no=240**		
Allele 2 no (%)	99 (41.2)	130 (54.2)	1.0	-
Allele 1 no (%)	141 (58.8)	110 (45.8)	1.68 (1.15 -2.46)	0.005 *

Table 4: IL1-ra genotype distribution between studied groups:

Groups / Alleles	Group I no= 240	Group II no =240	OR (95%CI)	p
Allele 5 no(%)	9 (3.8)	40 (16.7)	1.0	-
Allele 1 no(%)	72 (30)	48(20)	6.67 (2.79-16.33)	0.000**
Allele 2 no(%)	40 (16.7)	83 (34.6)	2.14 (0.89- 5.27)	0.06
Allele 3 no(%)	100 (41.7)	69 (28.8)	6.44 (2.78-15.3)	0.000**
Allele 4 no (%)	19 (7.9)	0 (0.0)	Undefined	0.000**

*P < 0.05, significant ** P < 0.001, highly significant

3.5. *Table (5) Relation of CIMT and parameters of RA disease activity and severity:* When comparing RA patients with CIMT ≥ 0.9 mm (67cases) and RA patients with CIMT<0.9 mm (53 cases), we found that the median of the MS, disease duration, swollen joints, tender joints, DAS28 and RF were significantly higher in the RA patients with CIMT ≥ 0.9mm than the others (P < 0.05). Also, the frequency of rheumatoid erosions was significantly different between the two sub-groups where it was higher in the RA patients with CIMT ≥ 0.9mm (P<0.05). However, there was no significant difference between the two sub-groups as regard the rheumatoid nodule (P > 0.05).

3.6. *Relation of CIMT and laboratory features of RA patients:* Table (6) showed that comparison between RA patients with CIMT ≥ 0.9mm (67cases) and RA patients CIMT<0.9mm (53 cases), there were highly significant difference between them as regard the median of the ESR (P < 0.001) and significant differences as regard cholesterol and triglycerides levels (P < 0.05). Where there were no significant differences between the two sub-groups as regards the CRP, fasting blood sugar, serum creatinine, BUN , ALT and AST.

3.7. *Table (7): Correlation between CIMT and the disease related variables in RA patients:*There were highly significant positive correlations between MS, disease duration, no of tender joints and CIMT (P < 0.001). Also there were statistically significant positive correlations between no of swollen joints, DAS28, ESR, RF, lipid profiles and CIMT (P < 0.05). While there were no significant correlations between CIMT and both factors (BMI& CRP) (P > 0.05).

Table 5: Relation of CIMT and parameters of RA disease activity and severity:

CIMT / Parameters	RA patients with CIMT<0.9mm no= 53	RA patients with CIMT ≥ 0.9mm no= 67	test	p
MS (min):				
median	10	15	Mann-whitney = 3.29	0.001**
range	10-120	10-60		
Disease duration(years):				
median	3	5	Mann-whitney = 3.8	0.000**
range	1-7	0.9-12		
Swollen joints: :				
median	2	4	Mann-whitney = 2.3	0.018*
range	1-9	1-10		
Tender joints: :				
median	4	6	Mann-Whitney= 4.5	0.000**
range	2-18	2-28		
DAS28				
median	4.5	4.8	Mann-whitney= 2.67	0.008*
range	3.1-6.7	3.4-7.8		
Rheumatoid nodule(no%)	15 (45.5))	18 (54.5)	$X^2 = 0.03$	0.86
Rheumatoid erosion(no%)	12 (30)	18 (70)	$X^2 = 4.9$	0.02*
Rhumatoid factor: median	37	150	Mann-whitney=2.1	0.03*

*P < 0.05, significant ** P < 0.001, highly significant Ms= morning stiffness DAS= diseas activity score CIMT= carotid intimal media thickness

Table 6: Relation of CIMT and laboratory features of RA patients.

Groups / Variables	CIMT< 0.9mm no= 53	CIMT ≥ 0.9mm no= 67	Mann-Whitney	p
ESR (mm/h):				
median	27	60	4.1	0.000**
-CRP (mg/ l):				
median	4.5	5.2	0.67	0.5
Lipid profiles (mg/dl):				
Total Cholesterol				
median	144	149	2.1	0.03*
Triglycerides				
median	65	80	2.4	0.01*
Fasting blood sugar(mg/dl):				
median	90	90	1.2	0.24
-Kidney function (mg/dl):				
Serum creatinine:				
median	0.6	0.6	0.82	0.41
BUN :				
median	21	23	0.14	0.89
-Liver function (U/L):				
ALT :				
median	21	14.9	1.8	0.07
AST :				
median	21.4	21.3	0.14	0.89

*P < 0.05, significant ** P < 0.001, highly significant Ms= morning stiffness CIMT: carotid intimal-media thickness ESR: Erythrocytes sedimentation rate. CRP: C- reactive protein BUN: Blood urea nitrogen. ALT:Alanine aminotransferase. AST: Aspartate aminotransferase.

Table 7: Correlation between CIMT and the disease related variables in RA patients:

Variables	CIMT	
	r	P
BMI	0.09	0.28
MS(min):	0.29	0.000 **
Disease duration(years):	0.33	0.000 **
No of swollen joints:	0.15	0.02 *
No of tender joints:	0.35	0.000 **
DAS28:	0.21	0.02 *
ESR (mm/hr)	0.31	0.01*
CRP (mg/l)	0.05	0.59
Rheumatoid factor(u/ml):	0.19	0.04*
Total cholesterol (mg/dl)	0.21	0.02*
Triglycerides (mg/dl)	0.3	0.01*

*P < 0.05, significant ** P < 0.001, highly significant BMI: body mass index Ms= morning stiffness DAS= disease activity score ESR: Erythrocytes sedimentation rate CRP= C- reactive protein.

3.8. *Synergistic effect of IL 1-β alleles carrier states on CIMT Table (8):*

Table 8: Synergistic effect of IL 1-β alleles carrier states on CIMT.

Risk allele	Groups	CIMT < 0.9mm	CIMT ≥ 0.9mm	OR (95%CI)	P
Allele 2	**control**	118	12	1.0	-
Allele 1	**control**	110	0	undefined	0.001*
Allele 2	**RA**	46	53	11.3(5.29-24.7)	0.000**
Allele 1	**RA**	60	81	13.2(6.4- 27.9)	0.000**

P < 0.05, significant ** P < 0.001, highly significant RA: Rheumatoid arthritis CIMT= carotid intimal-media thickness

It was found that RA patients who had the IL 1-β allele 1 showed an increased risk to develop atherosclerosis with CIMT ≥ 0.9mm (OR = 13.2, P = 0.000) compared with RA patients with IL 1-β allele 2 (OR = 11.3, P = 0.000). While in control there was no risk to develop atherosclerosis with IL1- β alleles.

3.9. *Synergistic effect of IL1-ra alleles carrier states on CIMT Table (9)*: It was found that RA patients who had the IL1-ra allele 1 showed an increased risk to develop atherosclerosis with CIMT ≥ 0.9mm (OR = 39, P = 0.000) compared with RA patients with other alleles of IL1-ra (OR = 14.3, P = 0.000) .While in control there were no risk to develop atherosclerosis with IL1-ra alleles.

Table 9: Synergistic effect of IL1-ra alleles carrier states on CIMT.

Risk allele	Groups	CIMT < 0.9mm	CIMT ≥ 0.9mm	OR (95%CI)	P
Other alleles	**Control**	180	12	1.0	-
Allele 1	**Control**	48	0	undefined	0.13
Other alleles	**RA**	86	82	14.3 (7.13-29.2)	0.000**
Allele 1	**RA**	20	52	39.0 (16.8-92.5)	0.000**

*P < 0.05, significant ** P < 0.001, highly significant RA: Rheumatoid arthritis CIMT= carotid intimal-media thickness.

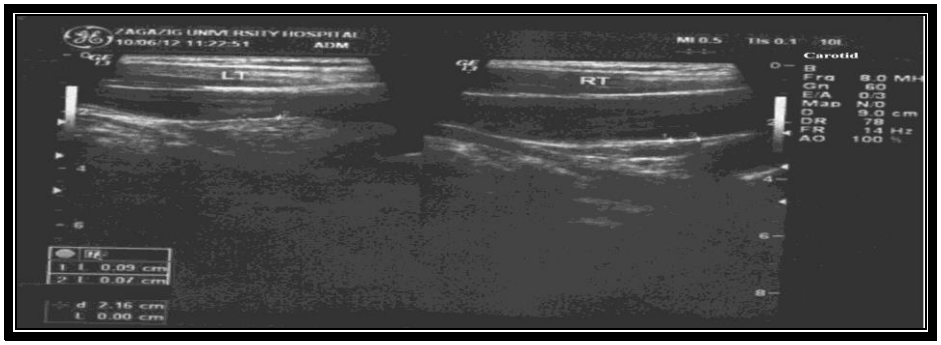

Fig. (1): B-mode ultrasonography showing intima-media thickness of the common carotid artery (0.76 mm in the left side and 0.9 mm in the right side).

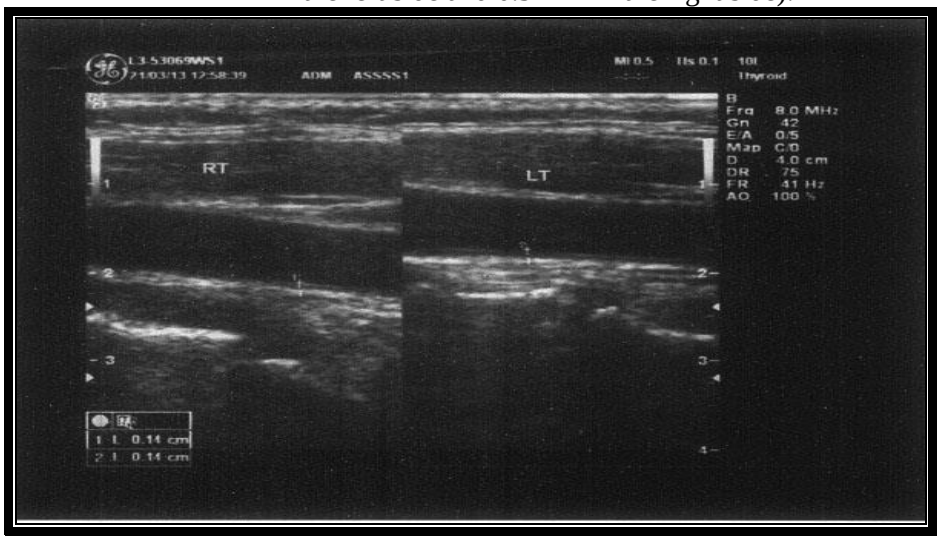

Fig. (2): B-mode ultrasonography showing intima-media thickness of the common carotid artery (1.4 mm in the left side and 1.4 mm in the right side).

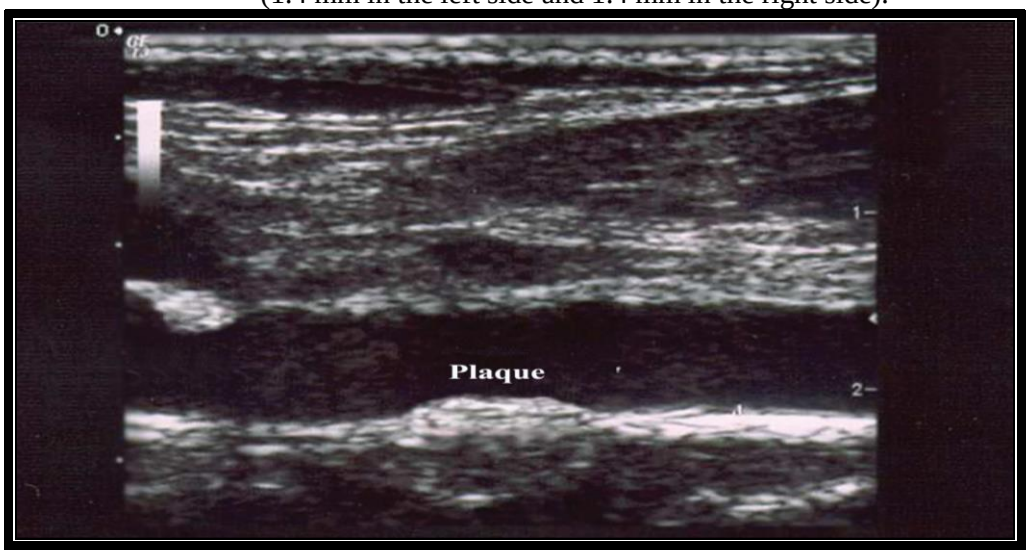

Fig. (3): B-mode ultrasonography showing soft tissue atheramtous plaque at the right common carotid artery.

4. DISCUSSION

In the present study, we found no significant difference between the two groups of the study as regards the age, sex, blood pressure and the body mass index (BMI).

As regards the laboratory variables between studied groups we found significant difference between the two groups of the study in erythrocyte sedimentation rate (ESR), C- reactive protein (CRP) and rheumatoid factor (RF). However, we found no significant difference between both of them as regards lipid profiles, fasting blood sugar, kidney function and liver function.

Our result matched with that of **AL-Tanawy et al., (9)** study; which included two groups; RA

patients and age and sex matched control where they found non-significant difference between the two groups as regards the age, blood pressure and the BMI, and they reported that no differences in the lipid profiles, fasting blood sugar, kidney function and liver function but as regard CRP there was significant difference between both of them.

In this work, we used the B-mode ultrasound for detection of atherosclerosis by evaluation the IMT and the plaques presence in the carotid arteries on both sides. We found highly significant increase in the thickness of the intima-media of the CCA on both sides and in the frequency of plaque occurrence in the RA patients than the control ($P < 0.001$).

These results were consistent with several results where study done by **Mahjan et al., (10)** found that there was a statistically highly significant difference between RA patients and controls regarding mean values of CIMT. Also they found that there was a high significant increase in the presence of plaques in RA patients than control ($P< 0.001$).

In our study, regarding allelic frequencies of the IL1- β polymorphism of patients with RA and controls, we found that the frequencies of allele 1 was significantly increased in patients with RA than controls [OR = 1.68, CI = (1.15 – 2.46), $P < 0.05$].

Our study were in agreement with the study done by **Chaudhary (11),** which was done on 100 Saudi patients with RA and 200 control and reported that frequencies of allele 1 was significantly increased in patients with RA than control ($P < 0.05$). The study concluded that IL1-β polymorphism is associated with susceptibility to RA and could be used as a prognostic marker for RA in ethnically Saudi RA patient.

Also, **Lee et al. (12)** compare different ethnic groups of RA patients using European, Asian, and Latin American population samples. Meta-analysis of the IL-1B genotype revealed, an association was found between the IL-1B polymorphism and RA susceptibility in Asian population only, while they found no association of this gene with other groups of patients.

The study done by **Arman et al., (13)** revealed that the frequency of allele 2 of IL1- β was significantly increased in Turkish patients with RA compared with control group (31.4% versus 20.2%), and subjects with the allele 1 were increased in healthy control. This differences may be explained by ethnicity which can greatly influence the genotype patterns between Egyptians and other groups of RA patients of different races.

In this study, comparison of the allelic frequencies of ILr-a between patients with RA and controls revealed that the frequencies of allel 1,3 were significantly increased compared to control group (allele 1 was 30.0% versus 20.0% and allele 3 was 41.% versus 28.8% respectively). Carriers of allele 1 and 3 were significantly more likely to develop RA (OR = 6.67, CI = 2.79-16.3, P = 0.000 and OR = 6.4, CI = 2.78–15.3, P = 0.000 respectively). Our study were in agreement with the study done on Spanish population by **Meulenbelt et al., (14)** and revealed that IL1-ra has a role in determine susceptibility to RA in those patients. Also, **Carreira et al., (15)** observed that the frequency of IL-1ra genotype in Spanish with RA was significantly different from the frequency in control ($P < 0.05$). It was found that L1-ra is an important genetic factor predisposing to susceptibility to RA in some ethnic groups that have exposure to specific environmental factors, but not in others, as this association has not been observed in Caucasian patients with RA who are of northern European origin **Lee et al., (12)**. In the current study, when comparing clinical and laboratory features of RA patients with CIMT<0.9 mm and those with CIMT ≥ 0.9mm revealed that RA patients with CIMT ≥ 0.9mm have significant increase in duration of MS, disease duration, no of swollen joints, no of tender joints, DAS28, frequency of erosions and levels of RF, ESR, cholesterol and triglycerides.

Our result matched with that of **Targońska-Stepniak et al., (16)** study which included 74 patients had RA & 31 control subjects where the risk factors for atherosclerosis were excluded. They reported the significant relation of these variables with carotid atherosclerosis in RA patients, even in the absence of traditional clinical CV risk factors, as RA exhibit premature atherosclerosis by way of increased CIMT and carotid plaques when compared to age and sex matched controls.

We did not find significant relation between the presence of rheumatoid nodules and increased thickness of the carotid artery as evidence of atherosclerosis in patients with RA; this result matched with that of **Angel et al., (17)** who reported that no association between rheumatoid nodules and the presence of CIMT in patients with RA. A possible explanation for the lack of association is that the pathogenic mechanisms involved in nodules formation, started by local trauma, permits the release of circulating immune complexes and RF from small vessels of

connective tissue, and that the macrophages and local monocytes activate and release cytokines and angiogenic agents.

In the present work, there were highly significant positive correlations between duration of MS, disease duration, no of tender joints and CIMT ($P < 0.001$). Also there were statistically significant positive correlations between no of swollen joints, DAS28, ESR, RF, lipid profiles and CIMT ($P < 0.05$).

These significant correlations between the common carotid IMT and disease activity in RA demonstrated in our study were in agreement with the results of **Van Doornum et al. (18)** who reported that the positive correlation between disease activity and severity of arterial stiffness supports the notion that chronic inflammation plays a role in RA associated atherosclerosis.Also, this result matched with that of **AL-Tanawy et al., (9**) study; which reported that there were statistically significant correlations ($p<0.05$) between IMT of CCA in RA and disease related variables; morning stiffness, duration of disease, pain severity, erosions and RF, and In the study done by **Rincon, et al (19)**, there was highly significant corelation between increased CIMT and DAS28 of RA patients. In the present work, there were statistically significant positive correlation between CIMT and lipid profiles (total cholesterol and triglyceride). This was in agreement with **Carotti et al., (20)** as there were statistically significant positive correlations between CCA-IMT and triglycerides and total cholesterol levels in the RA patients. Also, our results are consistent with those of **Jonsson, et al., (21)** who reported an association of increased CIMT with lipid profiles in RA patients.

In the current work, there were non-significant correlations between CIMT and BMI &CRP. In the study done by **AL-Tanawy et al., (9**) found no correlation between CIMT and BMI but there was a significant positive correlation existed between CCA-IMT and CRP. This difference in results may explained by elevation in the CRP level occur more with disease activity and the measurement of serum CRP at a single point failed to be associated with the IMT of the CCA.

In the present study, it was found that RA patients who had the IL 1-β allele 1 showed an increased risk to develop atherosclerosis with CIMT ≥ 0.9mm (OR = 13.2, P = 0.000) compared with RA patients with IL 1-β allele 2 (OR = 11.3, P = 0.000). Also, it was found that RA patients with the IL1-ra allele 1 showed an increased risk of CIMT ≥ 0.9mm than other alleles of IL1-ra (OR = 39, P = 0.000) compared with RA patients with other alleles of IL1-ra (OR = 14.3, P = 0.000). While in apparently healthy controls, there was no risk to develop atherosclerosis with either IL1- β alleles or IL1-ra alleles.

To the best of our knowledge, this is the first study discuss the relation between IL-1 ß alleles and IL-1 ß alleles with the development of atherosclerosis in rheumatoid arthritis patients. However as regard healthy controls the study done by **Zhou et al., (22)** found associations between IL-1 gene cluster polymorphisms and atherosclerosis in apparently healthy Chinese population, matched with our results as regard control group as there were no associations between IL-1 β and IL1-ra alleles gene cluster polymorphisms and atherosclerosis [(OR = 1.06, CI= 0.95–1.19), (OR = 1.00, CI= 0.85–1.17)] respectively. Study done by **Worrall et al.,(23)** suggested that allele 2 of the IL-1ra gene represents susceptibility factor in the development of carotid atherosclerosis in American population (OR =7.30; CI=2.3 to 22.9). As frequency of allele 2 was significantly greater in patients with atherosclerosis compared with nonatherosclerotic subjects in this study and Noncarriage of allele 2 was associated with reduced likelihood of atherosclerosis (OR=0.44; CI= 0.27 to 0.71), so suggested that allele 2 may represent a significant susceptibility gene in atherosclerotic development.

5. CONCLUSION

In conclusion, there was highly significant increase in the thickness of the intima-media of the common carotid arteries in the RA patients than control. Also, there was a highly significant difference in the frequency of plaques occurrence between both groups. our findings suggest that RA patients with the IL 1-β allele 1 increase risk of CIMT >13 folds more than other alleles and IL1-ra allele 1 increase risk of CIMT 39 folds more than other alleles. In conclusion IL 1-β and IL1-ra are emerging as novel common risk factors for atherosclerosis in RA patients.

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الملخص العربى

المقدمه والهدف من البحث:

إن مرض الرثيان المفصلي هو مرض مناعي وله أعراض إكلينيكيه ومناعية واسعة. مرض الرثيان المفصلي غير معروف السبب، ولكن تم التعرف على بعض التأثيرات الجينية الهامة التى لها دور فى حدوث المرض.

يزداد حدوث مرض تصلب الشرايين مع العديد من الأمراض المناعية الروماتيزمية و تزداد مخاطرمرض الشريان التاجي في المرضى الذين يعانون من الرثيان المفصلى.

وللتحورات الجينية المختلفة دور مهم فى الإصابة بتصلب الشرايين وأمراض القلب والأوعية الدموية .فلذلك تعتبر التأثيرات الجينية قاسم مشترك بين المرضى الذين يعانون من الرثيان المفصلى وتصلب الشرايين.

وقد كان الهدف من هذا البحث توضيح دور التحورات الجينية في الإنترلوكين ١ ومضادات مستقبلاته كعامل خطر محتمل بجانب عوامل الخطر الأخرى في حدوث تصلب الشرايين وأمراض القلب والأوعيه الدمويه فى مرضى الرثيان المفصلى. بالإضافة الى ايجاد العلاقة بين التحورات الجينية في الإنترلوكين ١ ومضادات مستقبلاته وكلا من حالة نشاط وشدة مرض الرثيان المفصلى.

المواد وطرق البحث:

يشتمل البحث على مجموعتين:-

المجموعة الأولى:- تتكون من ١٢٠ مريضه بالرثيان المفصلى يتواجدن بالعيادات الخارجية و القسم الداخلي لأمراض الروماتيزم والتأهيل بمستشفيات جامعة الزقازيق

والمجموعة الثانية:- الظابطه و تتكون من ١٢٠ من السيدات الأصحاء اللاتى يتوافقن في العمر والنوع مع مرضى الرثيان المفصلى.

وتم عمل الآتى للمرضى:

(١) التقييم إكلينيكي: وقد اشتمل على التاريخ المرضى الكامل ، فحص عام للمريض مع التركيزعلى الفحص المفصلي.

(٢) تقييم المرضى:ويتضمن تقدير درجة نشاط وشدة المرض لكل مريض تضمنه البحث.

(٣) عمل أشعه عاديه لليدين والرسغين (منظر خلفي أمامي)

وأجرينا الفحوص التاليه للمجموعتين:

(١) إستخلاص الحمض النووى للإنترلوكين ١.

(٢) إستخلاص الحمض النووى لمضادات مستقبلات الإنترلوكين ١.

(٣) نسبة الدهون (الكولسترول الكلي والدهون الثلاثية المستوى).

(٤) نسبة السكر الصائم في الدم.

(٥)اختبارات وظائف الكبد و الكلى.

(٦) عامل الروماتويد.

(٧) سرعة الترسيب وبروتين سي التفاعلى.

(٨) فحص الشريان السباتى على الجانبين الايمن والايسر بالموجات الفوق صوتية لقياس سمك الجدار المبطن للشريان السباتى المشترك وتقييم وجود لويحات في الشرايين السباتية المشتركة.

وقد تم الحصول على النتائج الأتية:

١) كان هناك فارق ذو دلاله إحصائية عالية بين مرضى التهاب الرثيان المفصلى و الأصحاء فيما يتعلق بمعدل سرعة الترسيب ، بروتين سى التفاعلى ونسبة عامل الروماتويد في الدم.

٢) وجد زيادة في سمك البطانة الوعائية لجدار الشريان السباتى المشترك وزيادة عدد لويحات الشريان السباتى في مرضى الرثيان المفصلى أكثرمن المجموعة الضابطة وكان الفرق ذو دلالة إحصائية واضحة.

٣) كشفت نتائجنا أن تعدد الأشكال الجينية للجين الوراثي الإنترلوكين ١ وخاصة الشكل الجينى أليل (١) له صلة ذات دلالة إحصائية بمعدلات حدوث مرض الرثيان المفصلي وحدتة.

٤) كشفت نتائجنا أن تعدد الأشكال الجينية للجين الوراثي مضادات مستقبلات الإنترلوكين ١ والشكل الجينى أليل (١) و أليل (٣) له دور فى التسبب فى مرض الرثيان المفصلى، بينما الشكل االجينى أليل (٤) لة علاقة بنشاط وشدة المرض.

٥) وكان هناك إرتباط ذو دلالة إحصائية فى زيادة سمك البطانة الوعائية لجدار الشريان السباتى المشترك ونشاط وشدة الرثيان المفصلى فى المرضى..

٦) فى مرضى الرثيان المفصلى كان هناك إرتباط ذو دلالة إحصائية بين سمك البطانة الوعائية لجدار الشريان السباتى المشترك إرتباطا إيجابيا مع مدة التيبس الصباحي للمفاصل، ومدة المرض،ومقياس شدة نشاط المرض ، عدد المفاصل المتورمة، ومستوى كلا من (سرعة الترسيب، عامل الروماتويد، ومستوى الكولستيرول و الدهون الثلاثية)فى الدم.

٧) وجد أن مرضى الرثيان المفصلى الذين لديهم الشكل الجينى أليل (١) للجين الوراثي الإنترلوكين ١ معرضيين إلى زيادة سمك البطانة الوعائية لجدار الشريان السباتى المشترك أكثر من أليل (٢) لهذا الجين.

٨) وجد أن مرضى الرثيان المفصلى الذين لديهم الشكل الجينى أليل (١) للجين الوراثي مضادات مستقبلات الإنترلوكين ١ معرضيين إلى زيادة سمك البطانة الوعائية لجدار الشريان السباتى المشترك أكثر من الأشكال الأخرى لهذا الجين.

الخاتمة:

- تزداد مخاطر القلب والأوعية الدموية وتصلب الشرايين زيادة سريرية في المرضى الذين يعانون من الرثيان المفصلى و ذلك يكون نتيجة عوامل خطر متعددة في هؤلاء المرضى.
- هناك زيادة فى سمك البطانة الوعائية لجدار الشريان السباتى المشترك وزيادة وتيرة حدوث لويحات الشريان الثباتى في مرضى الرثيان المفصلى.
- العلاقة بين التحورات الچينية في الإنترلوكين ١ ومضادات مستقبلاته وكلا من حالة نشاط وشدة المرض يثير احتمال دورها بوصفها عوامل الخطر لتصلب الشرايين في هؤلاء المرضى
- هناك زيادة فى معدلات حدوث زيادة سمك البطانة الوعائية لجدار الشريان السباتى المشترك المرضى الحاملين للأليل (١) لجين الإنترلوكين ١ وللأليل (١) لجين مضادات مستقبلات الإنترلوكين ١.