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Study of Interleukin 6 Promoter Gene Polymorphism in Egyptian Patients with Rheumatoid Arthritis and Its Correlation with Subclinical Atherosclerosis

Rania El-saied El-kholy[1*], Radwa M El-khouly[2], AE El-deeb[3], Amal M El-Barbary[4], Hanaa Hibishy Gaballah[5], Rasha Ahmed Gaber[6], Nagat M El-Gazzar[7]

ABSTRACT

The study was designed todetect IL-6 promoter gene polymorphism and its circulating level in rheumatoid arthritis (RA) patients and study its association with disease activity, severity of joint damage, and subclinical atherosclerosis.Patients and methods: Study included 60 RA patients and 40 matched healthy controls. Patients were assessed by disease activity score (DAS28), functional assessment using the Modified Health Assessment Questionnaire (MHAQ). Rheumatoid factor (RF), anti-cyclic citrullinated peptide (Anti CCP) antibodies, lipid profile and plasma IL-6 levels were measured. Genotyping for A -174G/C polymorphism of the IL-6 gene using polymerase chain reaction (PCR) technique was done. Musculoskeletal and subclinical atherosclerosis ultrasonographic assessments were applied.Results: Plasma IL-6 was found to be significantly increased in RA patients compared to the controls (p<0.001). IL-6 significantly correlated with clinical, laboratory and radiological parameters of disease activity and severity and subclinical atherosclerosis. The IL-6 -174 C allele was significantly higher in patients than controls (p=0.04). In patients with _174 CC genotype, there were significantly increased parameters of disease activity, severity and subclinical atherosclerosis.Conclusion:IL-6 has an essential role in synovitis, bone erosions and inflammatory systemic features; IL-6 −174G/C promoter polymorphism is associated with disease susceptibility, activity and severity. The increased cardiovascular disease (CVD) risk in RA patients appears to be autonomous of traditional cardiovascular risk factors.

Keywords: *Rheumatoid arthritis, ultrasound, interleukin-6, IL-6 −174G/C gene polymorphism, CVD.*

[1] *Physical Medicine, Rheumatology, and Rehabilitation Department, Faculty of Medicine, Tanta University, Tanta, Egypt.*
[2] *Physical Medicine, Rheumatology, and Rehabilitation Department, Faculty of Medicine, Tanta University, Tanta, Egypt.*
[3] *Physical Medicine, Rheumatology, and Rehabilitation Department, Faculty of Medicine, Tanta University, Tanta, Egypt.*
[4] *Physical Medicine, Rheumatology, and Rehabilitation Department, Faculty of Medicine, Tanta University, Tanta, Egypt.*
[5] *Medical BiochemistryDepartment, Faculty of Medicine, Tanta University, Tanta, Egypt.*
[6] *Medical BiochemistryDepartment, Faculty of Medicine, Tanta University, Tanta, Egypt.*
[7] *Physical Medicine, Rheumatology, and Rehabilitation Department, Faculty of Medicine, Tanta University, Tanta, Egypt.*

I. Introduction

Rheumatoid arthritis is a chronic, inflammatory, systemic autoimmune disease that may affect many tissues and organs, involving persistent joint synovitis, inflammatory cells infiltration, and overproduction of pro-inflammatory cytokines. It often causes serious joint deformity and even disability in the late period. [(1)] An increased risk of nearly all forms of CVD has been reported among RA patients. RA and atherosclerosis are inflammatory conditions of chronic nature that have similar pathophysiological mechanisms which present strong genetic susceptibility. Carotid artery intima-media thickness (CIMT) and brachial artery flow-mediated dilation percentage (FMD %) are useful tools used to detect subclinical atherosclerosis. [(2)]

IL-6 pathway has strong association with RA pathophysiology. It is present in the inflamed joints of RA patients and affects the function of multiple cell types including macrophages, T and B lymphocytes and osteoclasts.[(3)] Changes in IL-6 level are regulated by multiple factors, including the modulation of variations by polymorphisms within the promoter regions of the IL-6 gene, which have been associated with RA susceptibility in some populations. [(4)]

The extent of joint damage is highly variable feature among RA patients. The detection of erosive lesions in articulating surfaces by imaging techniques has a great diagnostic relevance and plays an important role in the early diagnosis of RA. [(5)] Musculoskeletal ultrasound (US) can detect early inflammatory soft tissue and bone destructive process in patients with normal radiologic and unclear clinical findings. [(6)]

II. Patients and methods:

This study was carried out on sixty RA patients selected from the outpatient clinics of Rheumatology and Rehabilitation department of Tanta University Hospitals. The patients fulfilled the ACR / EULAR 2010 classification criteria for RA [(7)] Forty apparently healthy nonsmoker volunteers matched with the patients group as regard age and sex were used as control group. Patients with history of atherosclerosis, smokers and those suffering from conditions that affect lipid profile as diabetes mellitus, hypothyroidism, liver or kidney disease, Cushing's syndrome, obesity (body mass index > 30) and familial dyslipidaemia were excluded. Informed consent was obtained from all subjects and the study was approved by the local ethics committee of faculty of medicine, Tanta University (30813/03/16).

Clinical assessment:Patients were subjected to full history taking, clinical examination, assessment of disease activity using DAS28, and functional assessment using MHAQ.

Laboratory evaluation: included RF, ESR, C reactive protein (CRP), Anti-CCP, total lipid profile. All participants underwent estimation of plasma IL-6 level by enzyme-linked immune sorbent assay (ELISA) technique using a commercially available kit (Catalogue number #: 201-12-0091) obtained from Sun Red Biotechnology Co., Shanghai, China. Supplied by Biokit Company, Egypt.

DNA extraction: Genomic DNA was extracted from the whole blood with EDTA using the Gene JET Whole Blood Genomic DNA Purification Mini Kit (#Cat. K0782, Thermo Scientific, Waltham, Massachusetts, USA). DNA purity and concentration were determined spectrophotometrically at 260 and 280 nm. The extracted DNA was stored at -20 °C until analysis.

Genotyping for A -174G/C polymorphism of the IL-6 gene:

Restriction fragment length polymerase chain reaction (PCR-RFL) was performed to determine the different genotypes of. Interleukin-6 (A -174G/C) polymorphism. This polymorphism was analyzed by amplification of a 611-bp sequence using oligonucleotide primer sequences designed according to Revilla et al.,2002 [8] as follows: the forward primer 5′- TGACTTCAGCTTTACTCTTGT-3′, and reverse primer 5′-CTGATTGGAAACCTTATTAAG-3′, these primer sequences are spanning interleukin-6 region containing A -174G/C polymorphism. Briefly, the protocol consisted of an initial denaturation at 94 °C for 4 min, followed by 30 cycles of annealing at 54 °C for 30 s, extension at 72 °C for 30 s and denaturation at 94 °C for 30 s, and a final extension at 72 °C for 4 min. The constituents of the reaction consisted of: 1.2 µM of each primer, 10 mM of dNTPs, 2 mM of MgCl2, 1 U of Taq DNA polymerase enzyme and 1× PCR buffer, along with 40–50 ng of DNA. All reactions were done using the thermal cycler Applied Biosystems 9600 (Per- kin Elmer, Singapore).

After amplification, the PCR product was digested with the restriction enzyme NlaIII (Thermo Scientific) in the manufacturer's buffer at 37 °C at for 4h. The products were then resolved on 3% agarose gel electrophoresis system and the bands were visualized with ethidium bromide staining under ultraviolet trans-illumination. 100 bp plus blue DNA ladder supplied from (#Calalog NO. GBR 104, Gene ON, Germany) ranged from (100 bp to3000 base pairs) was used to assess the size of the PCR-RFLP products. The amplified fragment (611 bp) after digestion with NlaIII restriction enzyme can give rise to either three fragments at 611, 367 and 244 bp, which indicates the presence of the heterozygous genotype (GC), or two fragments at 367 and 244 bp, which indicates the presence of the homozygous minor genotype (CC), or remains undigested as one fragment at 611 bp for the wild genotype (GG).

Radiographic assessment:

Musculoskeletal ultrasound assessment: by using the US7 Score [9] to assess erosions and disease activity.

Subclinical atherosclerosis detection by: (1) Measurement of IMT of common carotid artery using ultrasonography. [10] According to used sonographic criteria, when median CIMT was <0.9 mm, it was considered to be normal, while values ≥0.9 mm were considered to be an indicative of thickened intima and values >1.3 mm were considered to be indicative of atherosclerotic plaque. [11] (2) Ultrasound examination of brachial artery FMD. Subjects underwent noninvasive examination of endothelium-dependent vasodilation (FMD) of the brachial artery on the non-dominant arm, according to the International Brachial Artery Reactivity Task Force guidelines. [12] FMD was expressed as the relative increase in brachial artery diameter using hyperemia, and defined as (post hyperemic diameter – basal diameter)/basal diameter × 100. The maximum FMD diameter was calculated as the average of the 3 consecutive measurements and the percentage changes in the diameters compared with baseline resting diameter, and expressed as percentage diameter variation. In general, values of FMD% lower than 5-7% are considered abnormal.[12]

Statistical analysis of the data: Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**. (**Armonk, NY: IBM Corp**).** The statistical data are reported as the mean ± SE, frequencies and percentages when appropriate. Comparison between two means and more than two means was done using student t-test and ANOVA test respectively. On having significant ANOVA test, Tukey's HSD was done to find

out which specific groups's means (compared with each other) are different. Chi-square was used to examine the relationship between different variables. Pearson's correlation coefficient was used for detection of correlation between two quantitative variables in one group. A statistical significance was considered when $P \leq 0.05$

III. Results:

The characteristics of the patients and controls are presented in table 1. RA patients revealed mild dyslipidemia characterized by significant higher total cholesterol (TC), compared to controls. In addition, high-density lipoprotein cholesterol (HDL-C) levels were significantly lower compared to controls. As a consequence, the atherogenic ratio of TC/HDL-C and of LDL-C/HDL-C were significantly higher in RA patients compared to controls. There was significant increase of plasma IL-6 in RA patients compared to controls. ($P< 0.001$)

Table 1: Demographic, clinical data and laboratory investigations of the RA patients and control.

Parameters	RA patients (n = 60)	Control (n=40)	p
Age (years)	46.06 ± 10.77	44.2 ± 10.3	0.4
Sex M: F (n (%))	4/56 (6.7/93.3)	3/37 (7.5/92.5)	0.87
Duration of illness (years)	8.43 ± 6.67	-	-
Morning stiffness /min	87.6 ± 67.96	-	-
DAS28	4.67 ± 1.15	-	-
MHAQ	0.729 ± 0.58	-	-
ESR (mm/1sthr)	41.18 ± 20.2	19.75 ± 8.8	< 0.001*
CRP (mg/dl)	10.65 ± 5.7	2.22 ± 1.0	< 0.001*
RF (IU/ml)	56.87 ± 27.9	6.92 ± 3.3	<0.001*
Anti-CCP (U/ml)	218.1 ±108.8	10.75 ± 5.3	< 0.001*
TC (mg/dl)	172.3 ±18.6	155 ± 31.1	0.007
TG (mg/dl)	95.25 ± 32.4	87 ± 10.9	0.12
LDL –C(mg/dl)	93.5 ± 15.6	99 ± 15.8	0.08

HDL –C(mg/dl)	51.3 ± 8.1	77.6 ± 10.4	< 0.001*
LDL/HDL(mg/dl)	1.9 ± 0.5	1.07 ± 0.3	< 0.001*
TC/HDL(mg/dl)	3.36 ± 0.5	2.04 ± 0.5	< 0.001*
IL-6(pg/ml)	177.5 ± 79.3	16.9 ± 14.8	< 0.001*

* Significance $p \leq 0.05$. Values are expressed as Mean ± SD or n (%).Significance was determined using student t-test. DAS28; disease activity for 28 joint indices score, MHAQ; Modified Health Assessment Questionnaire, ESR ; Erythrocyte sedimentation rate, CRP; C-reactive protein, RF; Rheumatoid factor, Anti-CCP; anti-cyclic citrullinated peptide, TC; total cholesterol, TG ;triglycerides, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, IL-6;interleukin 6.

Table (2): Comparing genotype and allele frequencies of −174G/C polymorphisms of IL6 between rheumatoid arthritis patients and controls.

Genotyping	**RA patients (No.=60)**		**Controls (No.=40)**		**Chi -square**
	No.	**%**	**No.**	**%**	**p value**
GG	19	31.7	17	42.5	
GC	24	40	19	47.5	0.08
CC	17	28.3	4	10	
Total	60	100	40	100	
Allele					
G	62	51.7	53	66.25	0.04*
C	58	48.3	27	33.75	

Table (3): Genotyping in relation to different parameters in RA patients.

	GG	**GC**	**CC**	(p)
Parameter	**n = 19**	**n = 24**	**n = 17**	
Age (years)	44.2±10.8	45.9±11.1	48.4±10.4	0.5105
Duration of the disease (years)	6.4±5.3	7.9±5.5	11.5±8.6	0.0622
Morning stiffness	1.03±1	1.7±1.2	1.6±1.2	0.155
DAS28	4±1.1	5.09±1	4.84±1.1	0.005*****
MHAQ	0.45±0.5	0.72±0.5	1.04±0.6	0.008*****
ESR	38.7±18.6	40.91±21.5	44.29±20.9	0.7179
CRP	10.89±5.3	10.16±6.2	11.06±5.7	0.8686
Rheumatoid factor	55.1±27.1	58.66±27.8	56.2±30.4	0.917
Anti-CCP	213.9±112.9	216.8±124.3	224.64±83.9	0.955
TC	168.2±15.8	175.41±20.7	172.58±18.5	0.4574
TG	101.7±33.7	92.66±33.1	91.58±30.6	0.571
LDL - C	93.15±15.7	92.70±18.4	95±11.24	0.8955
HDL - C	50.84±7.8	52.66±7.5	49.88±9.5	0.546
LDL/HDL	1.88±0.47	1.78±0.42	1.99±0.57	0.4154
TC/HDL	3.36±0.5	3.33±0.45	3.4±0.6	0.918
IL-6	107.3±23.5	158.4±43.3	283.1±40.1	< 0.001*
GS_ synovitis (US7 score)	4.57±2.8	9.66±4.9	10.05±6.1	0.001*
PD_ synovitis(US7 score)	2.57±3.4	6.12±5.4	4.88±4.2	0.04*
GS_ tenosynovitis(US7 score)	±1.41	2.16±1.7	2.05±1.6	0.04*
PD_ tenosynovitis(US7 score)	1.26±1.8	3.83±3.3	3.88±3.4	0.01*

Erosion(US7 score)	1.73±1.4	6.12±5.1	7.47±5.2	< 0.001*
IMT	0.075±0.01	0.08±0.01	0.08±0.01	0.03*
FMD%	4.72±2.03	3.11±2.83	3.13±1.05	0.039*

Table (4): Correlation between interleukin-6 and different parameters in RA patients

Parameter	IL6 (pg/ml) in RA patients (n=60)	
	r	(p)
Age (years)	0.075	**0.56**
Duration of the disease (years)	0.17	**0.19**
Morning stiffness	0.42	**0.016***
DAS28	0.38	**0.019***
MHAQ	0.33	**0.022***
ESR	0.45	**0.012***
CRP	0.39	**0.02***
Rheumatoid factor	0.036	**0.785**
Anti-CCP	0.006	**0.967**
TC	0.080	**0.544**
TG	-0.050	**0.705**
LDL - C	0.047	**0.724**
HDL - C	-0.057	**0.665**
LDL/HDL	0.095	**0.469**
TC/HDL	0.069	**0.598**
GS_ synovitis (US7 score)	0.224	**0.086**

PD_ synovitis(US7 score)	0.551	**0.008***
GS_ tenosynovitis(US7 score)	0.038	**0.775**
PD_ tenosynovitis(US7 score)	0.089	**0.499**
Erosion(US7 score)	0.301	**0.02***
IMT	0.22	**0.047***
FMD%	-0.41	**0.017***

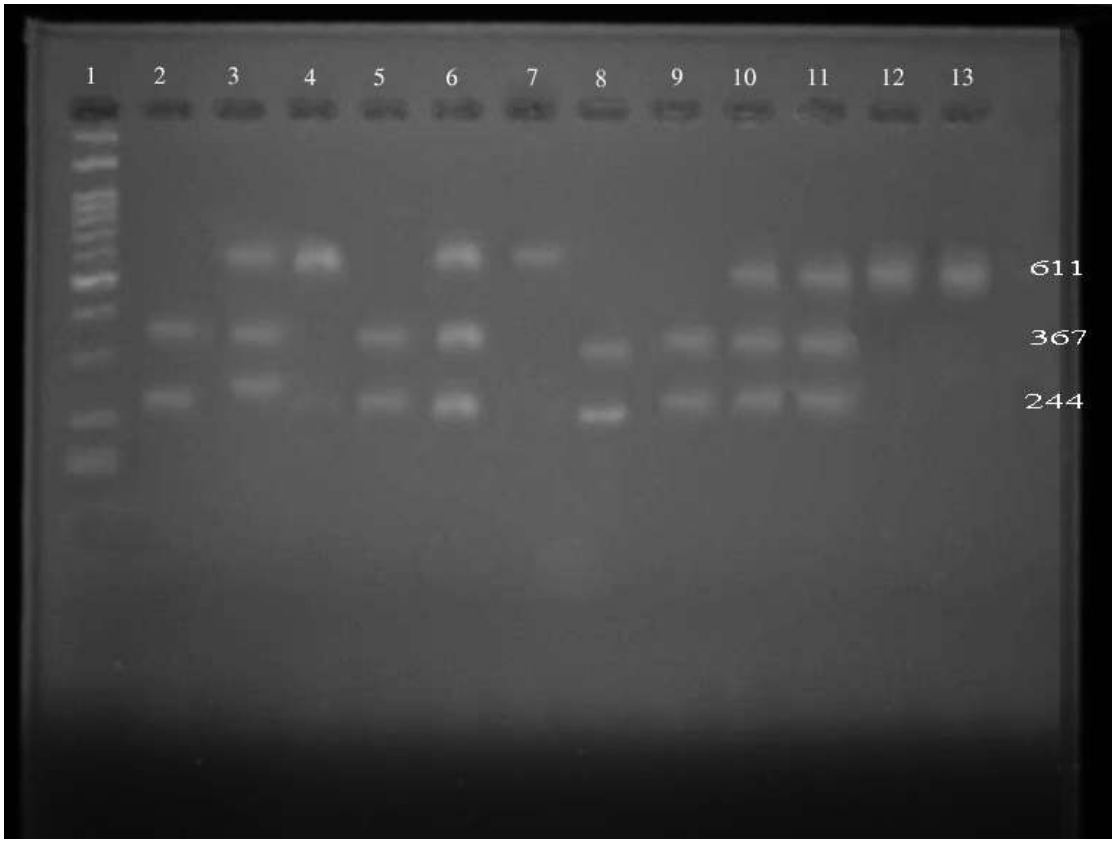

Figure (1): Genotyping of -174G/C IL6 gene polymorphism: Lane 1, 100 bp DNA molecular weight marker. Lane 2, 5, 8, 9 show 2 bands at 244, 367 bp denoting CC genotype. Lane 3, 6, 10, 11 show 3 bands 611, 367, 244 bp denoting GC genotype. Lane 4, 7, 12, 13 show one band at 611 denoting the wild genotype GG.

Comparing genotype and allele frequencies of −174G/C polymorphisms of IL6 between RA patients and controls are presented in table 2. The comparison of allele frequencies revealed that the polymorphic C allele was significantly frequent in RA patients compared with the control group. (P= 0.04)

Ultrasonographic assessment of patients was done by using the US7 Score to assess erosions and disease activity. The gray scale (GS)_ synovitis score mean was 8.17±5.3, the powered Doppler (PD)_ synovitis

mean was 4.65±4.7, GS_ tenosynovitis mean was 1.77±1.67, PD_ tenosynovitis mean was 3.03±3.2 and erosion was 5.12±4.9. 17 out of 60 RA patients (28.3%) had CIMT≥0.9 mm while 43 (71.7%) had normal CIMT<0.9 mm. The FMD% was less than 5% in 46 (76.7%) out of 60 RA patients while 14 (23.3%) had FMD% ≥ 5%.

Genotyping in relation to different parameters in RA patients are presented in table 3 .There was statistically significant difference in clinical and ultrasonographic values of disease activity, severity and CIMT and plasma IL 6 level among different genotypes and the values were significantly higher in CC & GC genotype when compared to GG genotype while the values of FMD% were lower in CC & GC genotype when compared to GG genotype. ($P \leq 0.05$)

Correlations between IL-6 and different disease parameters are presented in table 4. There was significant positive correlation between clinical, laboratory, radiological parameters of disease activity and severity and CIMT and plasma IL6 level while there was significant negative correlation between FMD% and plasma IL6 level.

IV. Discussion:

IL-6 gene is positioned on chromosome 7p21. There are numerous polymorphic sites found in the IL-6 gene promoter, among them; there is polymorphism 174G/C (rs1800795) suggested being associated with difference in cytokine production. This polymorphism is a single nucleotide change from guanine (G) to cytosine (C) at positions −174 in the promoter region [(8)].

In this study, RA patients showed significantly higher levels of total cholesterol, atherogenic index and significantly lower level of HDL–C in comparison to controls. A study by **Dessein et al**[(13)] has also reported lower levels of HDL-C and TC and higher TC/HDL-C and LDL-C/HDL-C ratios in active and/or untreated disease than in the general population.

In this study, the mean plasma IL-6 level was significantly higher in patients in comparison to control group. In agreement with our results, **Gaber et al, Chung et al, Wielińska et al**[(14-16)] found that serum concentration of IL-6 was significantly higher in patients when compared to healthy individuals. Also a study by **Nile et al**[(17)] suggested that higher levels of IL-6 were present in serum, synovial tissue and synovial fluid from RA patients compared to those with non-inflammed joints.

This finding supports the hypothesis that IL-6 cytokine has a role in the pathogenesis of RA as IL-6 promotes predominance of T helper 17 over T regulatory cells in the effector CD4+ T cell subsets, which is thought to play a major role in the development of RA and various other immune mediated diseases. In addition, IL-6 has been shown to promote T follicular helper cell development; which secretes IL-21; another B cell differentiation factor [(18)].

Most of patients had CIMT less than 0.9 mm (71.7%) while FMD% was less than 5% in 46 (76.7%). This is in agreement with **Signorelli et al** [(19)] who demonstrated that small proportion of the patients (7/30) exhibited abnormal CIMT values (≥0.9 mm) in young RA patients diagnosed with moderate disease duration, supporting the independent role of disease-related inflammation and immune dysregulation [(20)]. Altered function

of arterial wall has also been demonstrated in young RA patients with no history of CVD before treatment introduction and is thought to be the result of chronic inflammation and immune dysregulation [21].

In this study there was significant positive correlation of plasma IL6 level DAS28, laboratory (ESR, CRP) and US (PD_ synovitis) parameters of disease activity. This is in agreement with **Chung et al** [15] who found positive correlation between serum concentrations of IL-6 and CRP levels. A positive correlation between IL-6 concentration in RA patients and disease activity was also reported by **Raafat Hamed et al**[22]. In contrast **Wielinska et al**[16] did not observe any direct association between IL-6 serum levels and DAS28 in their cohort of RA patients. This disparity may be attributed to the differences in patients' origins and numbers of investigated cases or therapeutic agents used [23].

As regard disease severity there was significant positive correlation of plasma IL6 level with MHAQ, and erosions detected by ultrasonography. This is in agreement with **Knudsen et al**[24] who found that Plasma IL-6 among different inflammatory biomarkers was the only biomarker related to treatment response and progressive erosive disease in patients with early RA. IL-6 is a proinflammatory cytokine has a wide range of different activities which can mediate cartilage and bone damage, including induction of acute phase proteins and stimulation of T and B cells, synoviocytes and osteoclasts. It induces abnormal osteoclastogenesis in the inflamed joints of RA patients via the induction of RANKL expression in osteoblastic cells and synovial cells in the presence of intracellular adhesion molecule-1 [25].

In the present study there wasn't any correlation between plasma IL6 level and lipid profile however there was significant positive correlation of plasma IL6 level with IMT and significant negative correlation between its level and FMD%. This is in agreement with a study by **Dessein and Joffe** [26] who stated that reduced amount of circulating IL-6 cytokine was steadily associated with decreased endothelial activation, suggesting that suppression of IL-6 secretion lessens atherogenesis in RA.

Also **Hashizume et al**[27] have shown that IL-6 decreases levels of blood lipids by increasing the expression of the LDL receptor in different tissues. Moreover, RA has been established to be a risk factor by itself for increased IMT in a study conducted on female young RA patients with a favorable risk profile [28].

The results of the present study showed that the polymorphic C allele was significantly frequent in RA patients compared with the control group. This came in agreement with **Amr et al**, **Li et al** (2014a) and **Li et al** (2014b) [29-31] as they identified higher frequency of C allele in RA patients compared to controls. In contrast, **Palomino-Morales et al** [32] stated no significant differences in the IL-6 (174G/C) gene promoter polymorphism between RA patients and healthy individuals. Also **Marinou et al**[23] in United Kingdom reported that the frequency of the CC IL-6 (174G/C) gene promoter polymorphism was lower in RA patients than in the controls.

A meta-analysis study explained this divergence by the different genetic backgrounds. For instance, the -174C allele and its related genotypes were exceedingly lower in Asians and Eastern Chinese than in Europeans and other regions. Actually, it is now believed that one gene variant may play a different role in RA risk across different populations and regions [33].

As regard disease activity among different genotypes there was statistically significant difference and the values were higher in patients carrying C allele when compared to GG genotype. This is in agreement with **Wielinska et al**[16] who demonstrated more active disease in patients carrying C allele. In contrast, **Pawlik et**

al[34] identified those patients with a GG genotype have more active form of RA compared with CC and GC carriers and have more significantly increased parameters of disease activity (DAS28, ESR, and number of swollen and tender joints).

As for functional impairment assessed by MHAQ, there was significant difference among different genotypes where the values were higher in CC genotype when compared with GG genotype. This is in agreement with **Gaber et al**[14] who found that the more advanced the promoter polymorphism, the more the functional impairment of the patients.

In the present study the plasma IL 6 levels were significantly increased in GC& CC genotypes when compared to GG genotypes, and also in CC genotype when compared with GC genotype. In agreement with these results **Gaber et al** [14] stated that serum level of IL-6 was significantly correlated with its (-174G/C) promoter polymorphism and it was significantly higher in CC genotype over GC and GG genotypes carriers. Contrarily in a study by **Pascualet al** [35] -174 CC genotype was associated with less IL-6 serum levels than GC or GG genotypes carriers.

In the present study, when comparing IL-6 (-174G/C) promoter polymorphism types with patients' ultrasonographic finding by US7 score demonstrated higher values of bone erosions in CC genotype with the superiority of US over X-ray in assessing the soft tissues reflecting disease activity which also showed higher values in CC genotype. This is in agreement with the results of **Gaber et al**[14] who found that bone erosions were higher in those with CC genotype, but that was insignificant.

Similarly **Ceccarelli et al**[36] found that bone erosive damage demonstrated by US in Italian RA patients was significantly correlated with those with CC genotype. Also **Amr et al**[29] found significant association between the GC genotype and the C allele of the IL-6 -174G/C polymorphism, and severe joint radiographic damage in the hands of RA patients. In contrast **Marinou et al**[23] found that there is an association between the IL6-174G allele and increasing joint damage in RA patients.

The values of CIMT were significantly higher in CC genotypes when compared to other genotypes, with no significant difference in the lipid profile parameters among different genotypes. Similarly a study by **Panolous et al**[37] found that the association between IL6 -174C allele carriers and CVD in RA patients hasn't been related to traditional CVD risk factors, which denotes that the injurious effect of C-allele was exerted via its inflammatory pathways. But a study by **Palomino-Morales et al**[32] found that IL6_174 GG genotype RA patient's carriers had more severe endothelial dysfunction than GC or CC carriers.

The explanation of these controversial results of the above studies include the differences in study designs (different outcomes), the heterogeneity of the genetic structure of the studied populations (males only, elderly, patients carrying the risk genotype may have died earlier) and differences in the confounding factors that have been taken into account in the multivariable models.

A plausible explanation for these contradictory results may be that IL-6 has complex physiology. RA appears to be a polygenic disease and different genes may influence its phenotype and outcome. Also environmental factors, probably infectious agents, may induce difference in gene expression, which may be different according to the specific genetic background of the population, leading to different grade of the severity of the disease.

V. Conclusion:

IL-6 has an important role in synovitis, bone erosions and inflammatory systemic features. The increased CVD risk in RA patients appears to be autonomous of traditional cardiovascular risk factors.

The IL-6-174C-allele may associate with CVD in RA patients and possibly exerts its effect via increased inflammation. IL-6 −174G/C gene polymorphism is associated with disease susceptibility, activity and severity and also constitutes a genetic risk factor.

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