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Study the IL6 (C174G) Promoter SNP and Correlation with Physiological Growth Hormone and TNFA levels in Iraqi Subjects with Psoriasis

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ABSTRACT
Background: The chronic inflammatory skin disease with a strong genetic predisposition and autoimmune pathogenic traits is called Psoriasis.
Aim: Study the IL6 (C174G) promoter polymorphisms and correlation with growth factor and TNFA activity in patients with psoriasis in Iraqi population.
Subjects and Methods: This study is included 40 subjects (24 males and 16 females) with psoriasis at range of age (11-40) years with mean±SD was (29±11) years and divided into two groups. The control group included 20 subjects matched with age and gender of psoriasis group. GH and TNFA levels were estimated by use of ELISA technique. DNA was extracted from whole blood of both groups and PCR-RFLP was used to perform the genotyping analysis.
Results: The results suggesting highly significant differences in GH and TNFA levels between psoriasis and control group (p-value< 0.05). The results showing statistical differences (odd ratio) in CC, CG, and GG between psoriasis and control groups.
Conclusion: C174G polymorphism in promoter of IL6 gene with increasing in GH and TNFA levels consider a risk factor for incidence of psoriasis in different ages and genders.

Keywords: Psoriasis, IL6, Growth hormone, C174G SNP, TNFA

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INTRODUCTION
The chronic inflammatory skin disease with a strong genetic predisposition and autoimmune pathogenic traits is called Psoriasis. The prevalence in worldwide is about 2% but varies according to regions [1]. Psoriasis shows a lower prevalence in some populations such as Asian and African and increase to 11% in the populations such as Caucasian and Scandinavian [2,3]. The changing in dermatologic manifestations of psoriasis including psoriasis vulgaris is also called plaque-type psoriasis and is the most prevalent type. The scientific literature uses the terms psoriasis and psoriasis vulgaris to regard of; nonetheless, there are important distinctions among the different clinical subtypes [4]. The chronic plaque-type of psoriasis patients occurs in about 90% of all cases. Sharply demarcated, erythematous, and pruritic plaques covered in silvery scales are the main classical of clinical manifestations of psoriasis and the plaques can coalesce and cover large areas of skin. The trunk, the extensor surfaces of the limbs, and the scalp are common locations of psoriasis in the body [5-7]. One of the most of an important mediator of the acute phase response is Interleukin-6 (IL6), and its levels correlate with outcome from infection in a variety study [8]. The most common factor involved in this immune response is the pro-inflammatory cytokines, IL6 and tumor necrosis factor-A (TNFA) [9]. The major pro-inflammatory go between delivered by different cell types is IL6 that including melanoma cells, which applies diverse organic exercises towards an assortment of target cells (10). The fundamental factor that supposedly engaged with the separation of myeloid-derived silencer cells and the fortification of their suppressive capacity is IL6 and it is additionally connected with expanded creation of immunosuppressive cytokines by tumor cells, and expanded metastasis in melanoma (11,12). The major factor that play key roles in the regulation of cytokines expression is gene polymorphisms (SNP) [13]. The circulating levels of interleukin-6 can be affected by genetic polymorphisms in IL6 gene [14]. One of the most region has demonstrated to have a biological function in IL6 is the promoter region -174 of IL6 gene [15]. G allele of IL6 gene is associated with higher transcription activity than the C allele [16]. Studies on single nucleotide polymorphisms (SNPs) in the promoter region of IL6 gene in different Iraqi populations make more information on the role of this genetic variation in psoriasis susceptibility.

MATERIAL AND METHODS
Study design
This study is included 40 subjects (24 males and 16 females) with psoriasis at range of age (11-40) years with mean±SD was (29±11) years and divided into two groups. The control group included 20 subjects matched with age and gender of psoriasis group.
Determination of TNFA and GH levels
GH and TNFA levels were estimated by use of ELISA technique. The protocols were performed depending on instruction of manufactures.
Genotyping analysis
Genomic DNA was extracted from peripheral whole blood of all subjects (OS and NOS) who participating in this study by using the genomic DNA mini kit(Invitrogen™ PureLink™ Genomic DNA Mini Kit) that providing an efficient method for purifying of total DNA from whole and frozen blood. Allele specific PCR was performed by used unique primers for analysis of G/C IL6 genotyping and restriction enzyme (RE), as shown in table 1.

Table 1: primers and RE of IL6 gene that used in genotyping analysis

SNP	Primer sequence (5'→3')	Amplicon length	*Hin*I RE bands
C174G	F: TTGTCAAGACATGCCAAGTGCT R: GCCTGAGAGACATCTCCAGTCC	227 bp	118, 109 bp

PCR was carried out in a total volume 25 µl of reaction mixture with Taqman polymerase and carried by the thermocycler (bio rad) and subjected to denaturation at 95 Cº for 4 min, followed by 35 cycles of 95 Cº for 20 sec, 56.8 Cº for 1min and the final extension phase at 72 Cº for 5 min. The final PCR product was electrophoresis by agarose gel (2%) and photo documentation the products.

Statistical Analysis

Hardy Weinberg equilibrium was used to genotypes estimation. To determine the significant differences between the study groups as related with genotype and allele frequencies by using chi-square test in both patients and control groups.

RESULTS

Clinical characteristics of psoriasis group is shown in table 2:

Table 2: Characteristics of psoriasis group

CLINICAL VARIABLES	NO. TOTAL=30	PERCENTAGE (%)
Age		
11-25	**28**	**70**
26-40	**12**	**30**
Gender		
Male	**24**	**60**
Female	**16**	**40**
BMI		
≥30	**22**	**55**
<30	**18**	**45**

The results suggesting highly significant differences in GH and TNFA levels between psoriasis and control group (p-value< 0.05), as showing in table 3:

Table-3: TNFA and GH levels in study group

Groups	TNFA (pg/ml) mean± SD	P-value	GH (ng/ml) mean± SD	P-value
psoriasis n=40	**18.9±1.2**	**0.0000**	**150.9±17.8**	**0.0000**
Control n=20	**10.4±1.8**		**100.6±21.2**	

For genotyping analysis, PCR-RFLP used to amplification and restriction of target sequence of IL6 gene, as showing in figures-1 and 3:

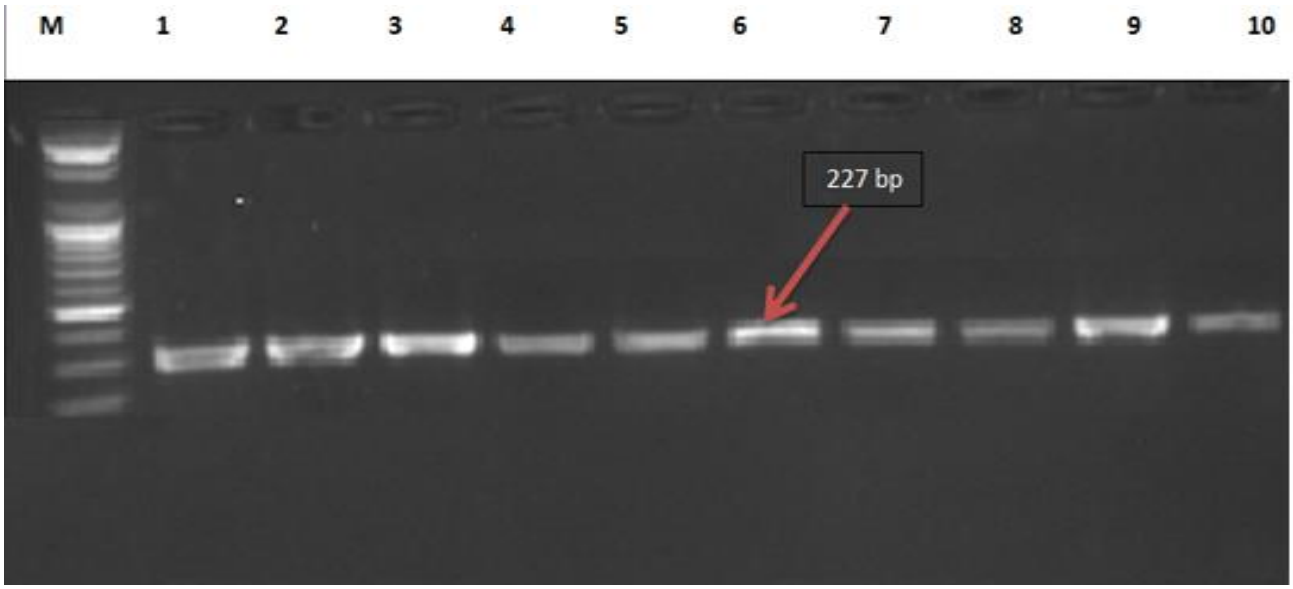

Figure 1: Electrophoretic pictures represents PCR amplification of IL6 gene of psoriasis group

The results suggesting statistical differences (odd ratio) in CC, CG, and GG between psoriasis and control groups, as showing in table 4:

Table 4: Comparison of three genotypes incidence in OS and NOS groups

GENOTYPES	PSORIASIS	CONTROL	ODD RATIO	CI 95%**
CC	21 (50%)	11 (37%)	2.543**	1.97-3.74
CG	11 (33%)	6 (33%)	1.418*	0.78-1.92
GG	8 (17%)	3 (30%)	1.785*	0.83-1.97
TOTAL	40(100%)	20(100%)	-	-

By application the data from the NCBI web site, the location of C174G SNP was determined, as showing in figure 2

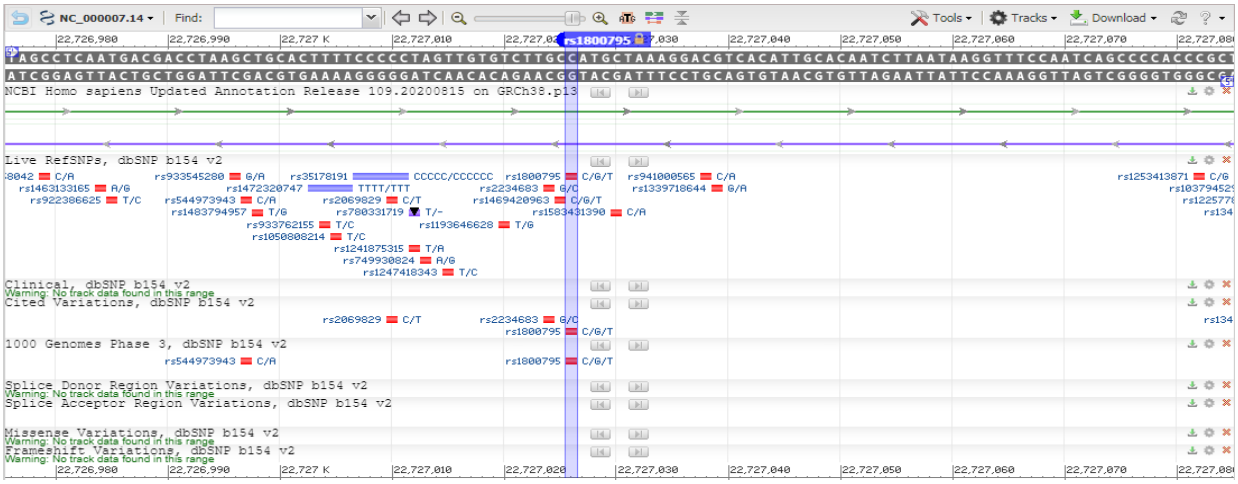

Figure 2: Location of 174 C/G promoter of IL6 gene on chromosome 7.

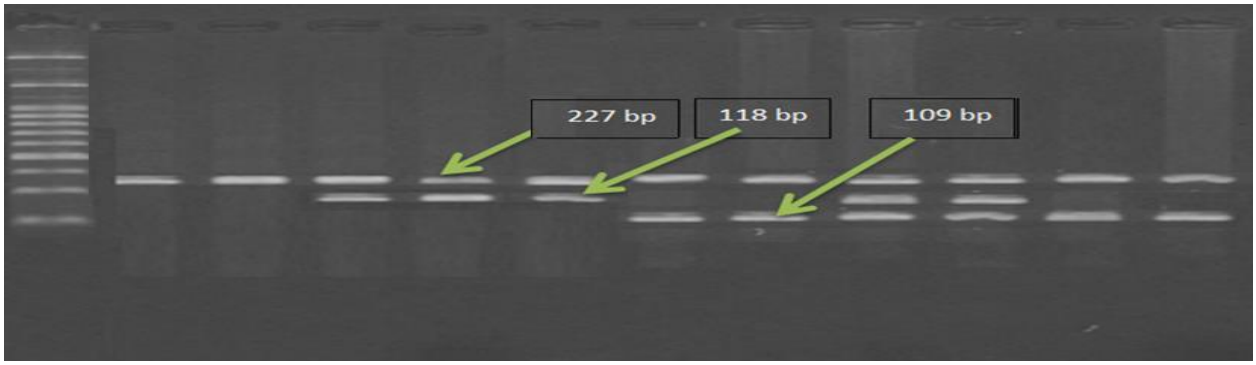

Figure 3: PCR-RFLP of 174 C/G promoter of IL6 gene showing three genotypes CC, CG, and GG in different

Bands length (227, 118, 109 bp).

The results suggesting the highly positive correlation (r^2=0.8895) between ages and levels of TNFA (pg/ml), but there was negative correlation(r2=0.358) with GH levels in of psoriasis group, as shows in figure-4:

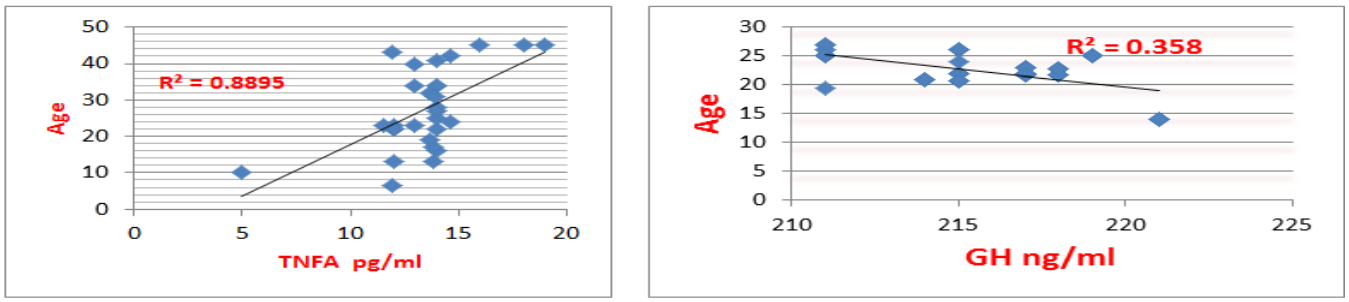

Figure 4: Correlation between ages with TNFA and GH levels in psoriasis group.

DISCUSSION

The main pleiotropic cytokine is IL6 that plays role in different immune disease but its role in the pathogenesis of psoriasis remains controversial. An interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine and in humans is IL6, it is encoded by the IL6 gene [17]. A polymorphism at position C174G of the IL6 promoter region is reportedly associated with different diseases such as inflammation in arthritis [18]. polymorphisms in the IL6 quality is hazard factor for the outflow of IL6. The IL6 C174G polymorphism is confined at the advertiser district of this quality and is related with adjusted advertiser action and coming about changing in protein articulation levels [19]. Fishman et al., 1998 were accounted for that 2-fold lower articulation in HeLa cells transfected with a vector containing the C allele of IL6 quality, contrasted and cells transfected with the G allele build [20]. Another in vitro examination likewise showed that the G allele of the IL6 C174GC SNP was related with an expanded transcriptional reaction to different upgrades [21].However, the results of studies investigating the levels of GH and TNFA and study the correlation with C174G polymorphism of IL6 the effects on are conflicting. The results suggesting highly significant differences in GH and TNFA levels between psoriasis and control group (p-value< 0.05). One o f t he most important pro-inflammatory cytokines that involved in cellular proliferation, differentiation and apoptosis, and has been reported to play a critical role in carcinogenesis is TNFA [22]. An association with mild liver disease, which correlates with IL6 polymorphism [23] and this may be explain the high levels of GH in psoriasis subjects .The genetic map of human of IL6 gene in chromosome was found to be mediated the insulin resistance and have a significant correlation and increased plasma IL6 levels with higher risk of Type2 diabetic mellitus (T2DM) making it is common gene [24] and this results agree with other study on ALKBH9 gene [25]. One of the main SNPs polymorphisms in the IL6 gene is in promoter (C174G) and it found to be regulated transcription in response to inflammatory stimuli such as lipopolysaccharides. Other studies reported that, IL6 promoter SNPs were considered as risk factors for T2DM development [26]. The results showing statistical differences (odd ratio) in CC, CG, and GG between psoriasis and control groups. IL-6 in turn stimulate the release of TNFA, thus perpetuating a vicious circle of chronic inflammation such as psoriasis. In conclusion, C174G polymorphism in promoter of IL6 gene with increasing in GH and TNFA levels consider a risk factor for incidence of psoriasis in different ages and genders.

CONFLICT OF INTEWREST

No potential conflict of interest relevant to this manuscript was reported.

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