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Genetic Single Nucleotide Polymorphisms of IL-16 and its Concentration of Patients Infected with Alopecia Areata in Diyala Province, Iraq

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Abstract: Alopecia areata is an autoimmune disease that causes hair loss all over the body, including the scalp, eyebrows, and beard. It was treated with immunosuppressants. Genetic factors play a significant role in alopecia areata infection. So, the current study aimed to investigate the genetic association of IL-16 single nucleotide polymorphisms (SNPs) with alopecia areata infections. The current study was conducted on two groups: alopecia areata patients' group included 51 individuals (involving 32 males and 19 females; with age means 27.90 ± 1.66 years), compared to a healthy control group consisting of 50 individuals (included 21 males and 29 females; the age means was 30.64 ± 2.08 years). IL-16 serum level was evaluated by using the ELISA technique, and the genetic polymorphism of IL-16 (rs11325 and rs1131445) was determined by sequencing the products resulting from the polymerase chain reaction technique. The present results revealed that the serum level of IL-16 was non-significantly increased in the alopecia areata patients group compared to the controls. In addition, IL-16 SNPs rs11325 genotypes and alleles frequencies showed that the GG genotype and *G* allele percentages were non-significantly increased in the patients' group (64.6% vs. 55.8% and 81.0% vs. 77%, respectively). Also, the results of rs1131445 recorded that the TC and CC genotypes and *C* allele frequency percentages were significantly increased in the patients' group (45.8% vs. 44.2%,16.7% vs. 14.0 and 40.0% vs. 36%, respectively). This study is the first of its kind which deals with the relationship between IL-16 levels and genetic polymorphisms with alopecia areata susceptibility among the Iraqi population, and Diyala governs. From the present findings, we can conclude that the TT genotype and *T* allele of the IL-16 SNPs rs11325 and TC, CC genotypes, and *C* allele of the IL-16 SNPs rs1131445 have a role as relative risks for alopecia areata. More genetic studies should be conducted to ensure the role of cytokines in alopecia areata susceptibility.

Keywords: Alopecia areata, ELISA, PCR, DNA, IL-16, SNPs, Polymorphism

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Introduction

Alopecia areata (AA) is an autoimmune disease resulting from immune system cells attacking the hair follicles, leading to hair loss that ranges in appearance from circular patches on the scalp to total scalp or body hair loss (Putterman *et al*., 2019). Alopecia areata is the second most common cause of hair loss after androgenetic alopecia, affecting 2% of the global population, with increasing prevalence (Mane *et al*., 2011). Acute alopecia areata or some clinical subtypes are rare, with the prevalence of alopecia areata, alopecia totalis, and alopecia universalis being 0.02%, 0.08%, and 0.03%, respectively (Ahmed *et al*., 2010). AA affects both sexes equally at different age periods. It has no specific age, gender, or ethnic preferences. The prevalence of AA is higher in children and adolescents than in adults (Mane *et al*., 2011). Several studies have confirmed an association between IL-16 and autoimmune diseases. Lee *et al*. (2014) found the first association between IL-16 and systemic lupus erythematosus (SLE). They recorded a significant increase in the level of IL-16 in the sera of SLE patients; also reported an association between IL-16 serum level and disease severity. Also, Tanyasiri *et al*. (2005) found a significantly increased level of IL-16 in the serum of patients with alopecia areata. Several studies reported a relationship between the disease and the immune system by inducing the T lymphocytes, especially CD8 and CD4 T lymphocytes, resulting in increasing the production of IL-16, a soluble molecule that ligands the CD_4 T lymphocytes as antigenic stimulator (Center and Kornfeld, 1996; Sekigawa *et al*., 2000; Cruikshank and Little, 2008). IL-16 is a gene located on chromosome 15q26.3 of humans and contains 25 exon-encoded regions, denoted by HGNC and known as LCF; NIL16; PRIL16; prIL- 16 (Kim, 1999; NCBI, 2021), consists of seven exons and six introns (Gilhar *et al*., 1999). Genetic factors play a significant role in alopecia areata infection.

Hence, the present study was aimed to investigate the genetic association of IL-16 single nucleotide polymorphisms (SNPs) and its serum level with alopecia areata infections.

Materials and Methods

Collection of blood samples:

Fifty-one cases were collected from patients with alopecia areata (32 males and 19 females) when they visited the health center for diagnosis and treatment, the age mean of the alopecia areata (AA) group was 27.90 ± 1.66 years, compared to 50 healthy controls (21 males and 29 females), their age mean was 30.64 ± 2.08 years. The blood samples were collected from the health care center at Diyala province from 14th December 2021 to 15th June 2022. The blood samples were collected in EDTA tubes, then all samples were kept by freezing at -20˚C until the DNA extraction process began. The written agreement was obtained from all the participants through the questionnaire, including their age, gender, living location, and other questions related to the disease. Also, the ethical approval was obtained from the Ethical Approval Committee of the University of Baghdad, College of Science (CSEC/0322/0151).

The serum level of IL-16 was measured using the ELISA technique following the manufacturer's protocol (Al-Shukairat Company, Jordan).

Primer's preparation:

The primer was designed using the National Center for Biotechnology Information (NCBI) website for conducting molecular detection and determining the polymorphism of the IL-16 gene, as shown in Table 1.

DNA extraction and amplification:

Genomic DNA was extracted from EDTA blood samples of alopecia areata patients and healthy control groups using the commercial DNA Purification Kit according to the manufacturer's protocol (Favorgen, Taiwan). The extracted DNA was transferred on 1% agarose gel with an electric potential difference of 100 volts /30 min. The

Table 1: The primers information of IL-16 gene polymorphisms

Interleukin	Primer	Sequence (5`-3`)	Product length
IL-16	**Forward primer**	**TCATCTGTGGAACCCATTAC**	963 bp
	Reverse primer	**GGTTTGTGTCAGCTCTAACT**	

thermocycling conditions were: initial denaturation at 95 ºC for 5 min/1 cycle, followed by 35 cycles, including denaturation at 95 ºC for 30 sec, annealing at 62 ºC for 30 sec, and extension at 72 ºC for 30ºC sec, then 1 cycle of final extension at 72 ºC for 10 min, transferred to electrophoresed on 1.5% agarose gel with an electric potential difference of 90 volts/60 min after staining with ethidium bromide and photographed using ultraviolet light transilluminator. The amplicon of the IL-16 genetic polymorphisms was sent to Macrogen company in South Korea to determine the nucleotides sequences, and to compare the obtained nucleotides sequences with the nucleotides sequences of the same gene found in the National Center for Biotechnology Information (NCBI) gene bank using the Geneious program, for determining the IL-16 genotypes of each sample were determined.

Statistical analysis:

IBM SPSS computer program version 27.0 was used to analyze the data statistically. The normality, homogeneity, and randomization were calculated before the statistical analysis of the data began. The mean and standard error was calculated for the age and the serum level of IL-16, independent t-test and ANOVA table (Tukey test) were used to compare the studied group at a significance level less than 0.05. Genotypes of IL-16 SNP were presented as frequencies' percentages between their distributions in alopecia areata patients and controls. Fisher's exact probability (2- tailed) was used to assess the probability. In addition, the odd ratio (OR) and 95% confidence intervals were estimated to determine the relative and the preventive fraction to determine the association between the genotypes and the disease development. The probability of these estimations was calculated using Pearson chi- square. Also, the Hardy-Weinberg equilibrium calculator was used to compare the observed and expected observations.

Results

The current study included two groups; the first included 51 patients (32 males and 19 females), and the second included 50 healthy control individuals (21 males and 29 females) and their ages ranged from less than 10 years to 60 years. The age mean of the AA group was (27.90 ± 1.66) years, while the age mean of the control group was 30.64 ± 2.08 years, there was no significant difference appeared between the groups. The gender percentage in the AA group showed a significantly increasing frequency ($P < 0.05$) in the infected males than the infected females (62.7% *vs.* 37.3%, respectively) compared to 42.0% for males and 58.0% for females in controls. Also, 68.6% of AA patients lived in urban compared to 52.0% of controls, while the infection rate among the rural population was 45.10%, compared to the control group 48.00%, this did not constitute a significant difference at a probability of $P > 0.05$, with a mean incidence of infection (78.98±38.46) weeks. (Table 2).

IL-16 serum level showed a non-significant increase in the AA patients' group compared to the control group (214.56 ± 22.48 *vs.* 167.07 ± 23.59 pg/ml) (Table 2).

The extracted DNA from the AA patients and control groups was electrophoresed on 1% of

Table 2: The number and living and age and IL-16 level mean and standarderror of the AA patients' group compared to the controls

Parameters	Patients group	Control group	Probability
Male No.(%)	32(62.70)	21(42.00)	0.037
Female NO.(%)	19(37.30)	29(58.00)	
Living Urban No.(%)	28(54.9)	26(52.00)	0.77
Living Rural No.(%)	23(45.10)	24(48.0)	
Age Means level ± SE(Years)	27.90 ± 1.66	30.64 ± 2.08	0.305
Infection period Means level ± SE	78.98±38.46	0.00±0.00	$1.30x10^{-7}$
IL – 16 Means level ± SE (pg/ml)	214.56 ± 22.48	167.07 ± 23.59	0.154

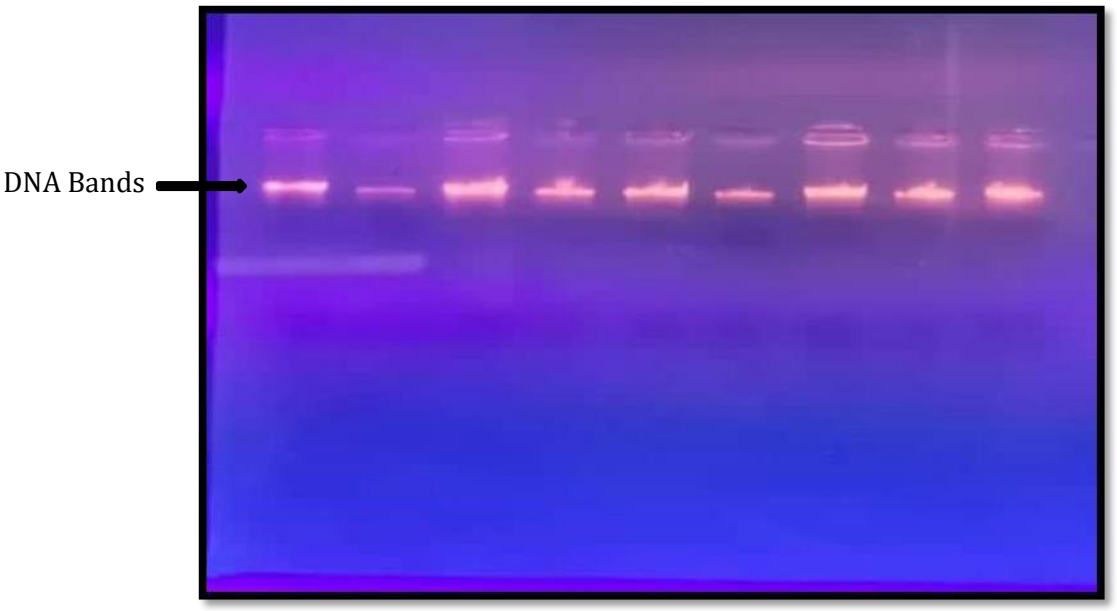


Fig. 1: Electrophoresis of chromosomal DNA bundles extracted from blood samples of alopecia areata patients and healthy subjects and carried over on agarose gel with a concentration of 1% and an electric potential difference of100 volts/30 min.

agarose gel at an electric potential difference of 100 volts/30 min (Fig. 1) The purity and concentration of the yielded DNA were measured using a nanodrop device, the purity ranged between 1.7 - 2.0 nm, and the concentration ranged between 50 – 100 pmol/ml. This purity is required in the polymerase chain reaction (PCR).

The amplification results of the IL-16 gene showed that the molecular size of the electrophoresed amplicons bands was 963 bp when electrophoresed on 1.5 % agarose gel stained with ethidium bromide (Fig. 2).

The molecular analysis of IL-16 genetic polymorphism showed a variation in two sites: rs11325 G > T on chromosome Chr15:81308999 for nucleotide 117122 of the downstream variant sequences, and rs1131445 T > C on chromosome Chr15:81309441 for nucleotide 117564 for the non-coding transcript variant, and T>C rs1131445 on chromosome 15:81309441 of non-coding transcript variant of nucleotide location at 117564 in the studied samples. This variation is a point

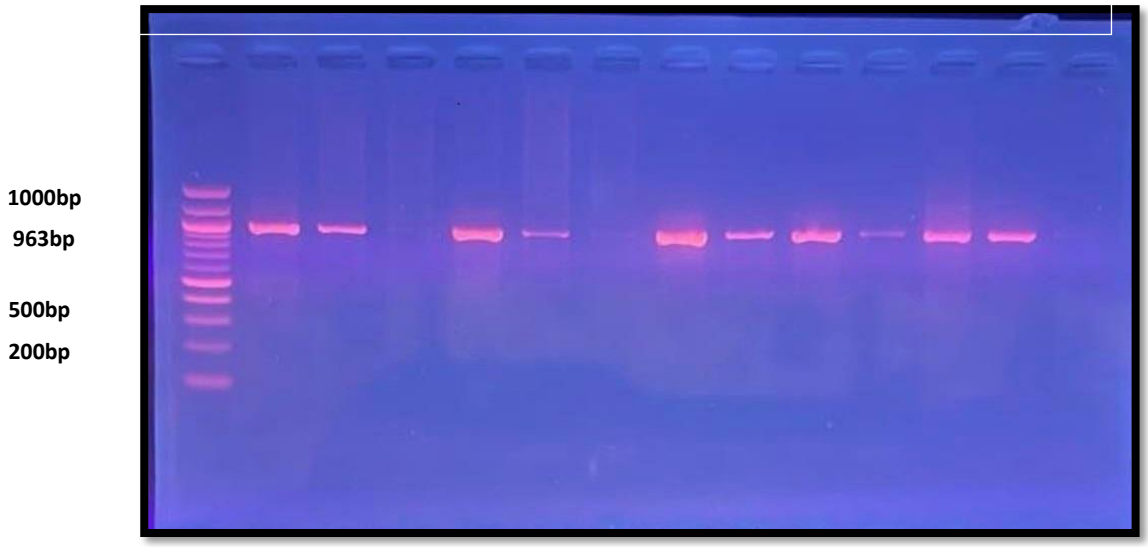
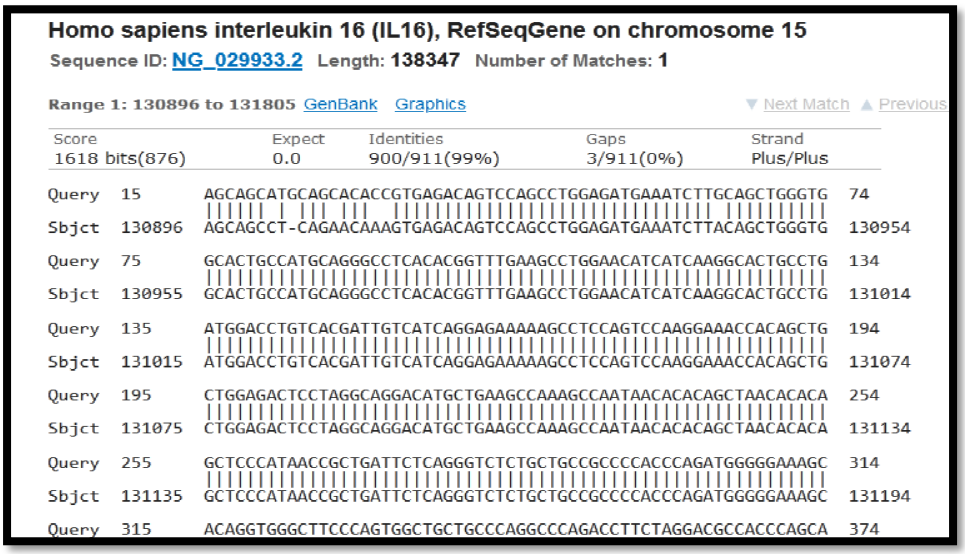


Fig. 2: The amplification product of the IL-16 gene carried on 2% agarose gel at a voltage difference of 90 volt for 60 min stained with ethidium bromide and photographed with an ultraviolet light transilluminator. The molecular size of the appeared bands was 963 bp.



Homo sapiens interleukin 16 (IL16), RefSeqGene on chromosome 15

Sequence ID: NG_029933.2 Length: 138347 Number of Matches: 1

Range 1: 130896 to 131805 GenBank Graphics ▼ Next Match ▲ Previous

Score	Expect	Identities	Gaps	Strand
1618 bits(876)	0.0	900/911(99%)	3/911(0%)	Plus/Plus

```
Query  15      AGCAGCATGCAGCACACCGTGAGACAGTCCAGCCTGGAGATGAAATCTTGCAGCTGGGTG  74
               ||||||  | |||  |||  ||||||||||||||||||||||||||||| ||||||||||
Sbjct  130896  AGCAGCCT-CAGAACAAAGTGAGACAGTCCAGCCTGGAGATGAAATCTTACAGCTGGGTG  130954

Query  75      GCACTGCCATGCAGGGCCTCACACGGTTTGAAGCCTGGAACATCATCAAGGCACTGCCTG  134
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  130955  GCACTGCCATGCAGGGCCTCACACGGTTTGAAGCCTGGAACATCATCAAGGCACTGCCTG  131014

Query  135     ATGGACCTGTCACGATTGTCATCAGGAGAAAAAGCCTCCAGTCCAAGGAAACCACAGCTG  194
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  131015  ATGGACCTGTCACGATTGTCATCAGGAGAAAAAGCCTCCAGTCCAAGGAAACCACAGCTG  131074

Query  195     CTGGAGACTCCTAGGCAGGACATGCTGAAGCCAAAGCCAATAACACACAGCTAACACACA  254
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  131075  CTGGAGACTCCTAGGCAGGACATGCTGAAGCCAAAGCCAATAACACACAGCTAACACACA  131134

Query  255     GCTCCCATAACCGCTGATTCTCAGGGTCTCTGCTGCCGCCCCACCCAGATGGGGGAAAGC  314
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  131135  GCTCCCATAACCGCTGATTCTCAGGGTCTCTGCTGCCGCCCCACCCAGATGGGGGAAAGC  131194

Query  315     ACAGGTGGGCTTCCCAGTGGCTGCTGCCCAGGCCCAGACCTTCTAGGACGCCACCCAGCA  374
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Fig. 3: Comparison of the sequence match of a portion of the IL-16 gene of samples of patients with alopecia areata with that of the Gen-bank sample (NCBI).

mutation of the substitution type located at a specific location in the nucleotide sequence.

The DNA sequencing of the IL-16 selected gene's region showed that two samples of the AA patients' group and seven samples of the healthy control were discarded because rubbish results appeared when these samples were sequenced. The sequences of 48 samples of alopecia areata patients and 43 samples of healthy people were compared with each other in one chart for the purpose of investigating the variations within the 963bp segment of the variant loci of the IL-16SNPs gene included (rs11235 and rs1131445) (Fig. 3). This is what is known as sequence alignment, as the program used in this alignment is Genius, that this alignment is the method used to know any nucleotide variation and its location within that sequence, as the comparison of those sequences with each other and the presence of Sequencing the reference DNA (original) and comparing the DNA of those samples with the stored reference sequence (Gene ID: 029933.2).

Table 3: Distribution of genotypes and allelic frequency of the IL-16 gene at the rs113225G>T heterogeneity site in the study groups according to the Hardy-Weinberg equilibrium law in alopecia areata patients compared to the control in Gene ID: 117122

Genotyping of IL-16 SNPs rs11325	**Patients group No. (%)**		**Control group No. (%)**	
	Observed	**Expected**	**Observed**	**Expected**
GG	31 (64.6)	31.7 (66.0)	24 (55.8)	25.3 (58.9)
GT	16 (33.3)	14.6 (30.5)	18 (41.9)	15.3 (35.7)
TT	1 (2.1)	1.7 (3.5)	1 (2.3)	2.3 (5.4)
Total	48 (100.0)	48 (100.0)	43 (100.0)	43 (100.0)
***P*-HWE**	0.5148		0.2574	

P-HWE: the probability of Hardy-Weinberg equilibrium

Table 4: Evaluation of the relationship between the genotype and alleles of the IL-16 gene at the site of the variation G>T rs11325 in the studied samples

Genotyping of IL-16 SNPs rs11325	**Patients group No. (%)**	**Control group No. (%)**	**OR**	**95% CI**	**Fisher's exact probability**
GG	31 (64.6)	24 (55.8)	1.44	0.63 – 3.33	0.520
GT	16 (33.3)	18 (41.9)	0.69	0.30 – 1.61	0.515
TT	1 (2.1)	1 (2.3)	0.89	0.06 – 14.29	1.0
Total	48 (100.0)	43 (100.0)			
Alleles frequency					
G	78 (81.0)	66 (77.0)	1.31	0.64 – 2.68	0.471
T	18 (19.0)	20 (23.0)	0.76	0.37 – 1.55	0.471

OR: odd ratio, 95% CI: 95% confidence intervals

For IL-16 rs11325 genotyping and alleles frequencies, the single nucleotide polymorphisms (SNPs) investigated two alleles, *G* and *T* corresponded to three genotypes (GG, GT, and TT), where no significant difference was observed between the observed and expected frequencies of the three genotypes, and compatible with Hardy-Weinberg equilibrium (Table 3). The GG genotype and *G* allele were non- significantly increased frequency percentage in the AA patients' group compared to the healthy control group (64.6% *vs.* 55.8 %, OR: 1.44, P = 0.520; and 81.0% *vs.* 77.0%, OR: 1.31, P = 0.471, respectively) (Table 3). Also, the high OR value of GG genotype and *G* allele referred that this genotype and allele might be a relative fraction and have an etiological agent for alopecia areata (1.44, 1.31, respectively) (Table 4). While the frequency percentage of the GT and TT genotypes and *T* allele were non-significantly decreased in the AA patients' group than in the control group (33.3% *vs*. 41.9%, 2.1% *vs*. 2.3%, and 19.0% *vs.* 23.0%, respectively) (Table 4). The low OR value of GT, TT genotypes and *T* alleles might be considered a preventive fraction from alopecia areata development (0.69, 0.89 and 0.76, respectively).

Figure 4 shows the alignment of the nitrogenous bases for part of the IL-16 gene between the AA patients' group and the healthy control group, explaining the location of the rs11325 variation between the sequence of the nitrogenous bases. That is an inversion and transition between bases by replacing the G (CGG)

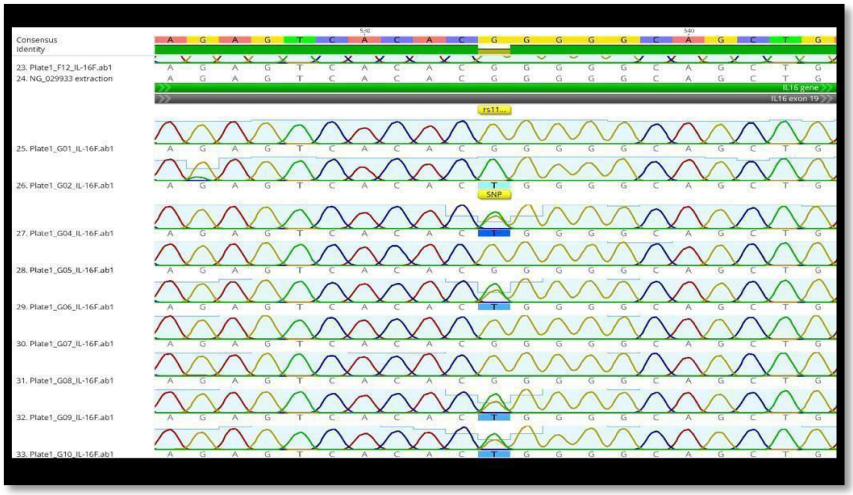

Fig. 4: The alignment of the nitrogenous bases of a part of the IL-16 gene between alopeciaareata patients and a control group showing the location of the IL-16 SNPs rs11325.

Table 5: Distribution of genotypes and allelic frequency of the IL-16 gene at the heterozygous site rs113225 T>C in study groups according to the Hardy-Weinberg equilibrium law in alopecia areata patients compared to control in Gene ID:117564

Genotyping of IL16SNP rs1131445	**Patients group No. (%)**		**Control group No. (%)**	
	Observed	Expected	Observed	**Expected**
TT	18 (37.5)	17.5 (36.5)	18 (41.9)	**17.6 (40.9)**
TC	22 (45.8)	23.0 (47.8)	19 (44.2)	**19.8 (46.1)**
CC	8 (16.7)	7.5 (15.7)	6 (14.0)	**5.6 (13.0)**
Total	48 (100.0)	48 (100.0)	43 (100.0)	**43 (100.0)**
P-HWE	0.7724		**0.7848**	

P-HWE: the probability of Hardy-Weinberg equilibrium

with another T (CTG) which results in a substitution mutation leading to a change in the amino acid code, and thus arginine turns into leucine.

For IL-16 SNPs rs1131445, the findings of genotyping and alleles frequencies' percentages appeared that the genotypes of both studied groups (the AA and the control groups) were investigated into two alleles *T* and *C,* which corresponded to three genotypes (TT, TC, and CC), which showed no significant difference between the observed and expected frequencies of the three genotypes with compatibly to the Hardy-Weinberg equilibrium (Table 5). The TT genotype and *T* allele were non-significantly increased frequency percentage in the AA patients' group compared to the healthy control group (37.5% vs. 41.9 %, OR: 0.83, p = 0.830; 60.0% vs. 64.0%, OR: 0.86, p = 0.649, respectively) (Table 5). The low OR value of the TT genotype and *T* allele might be suggested to have a preventive fraction from alopecia areata (0.83, 0.86, respectively) (Table 6). The results of TC and CC genotyping percentages showed a non-significantly increase in the AA patients' group compared to the control group (45.8% *vs.* 44.2% and 16.7% *vs.* 14.0%, respectively). Also, the OR value of these genotypes was 1.07 and 1.23, respectively (Table 6).

Figure 5 illustrates the alignment of the

Table 6: Evaluation of the relationship between the genotype and alleles of the IL-16 gene at the site of the variation rs113445 in the studied samples

Genotyping of IL16SNP rs1131445	Patients groupNo. (%)	Control groupNo. (%)	OR	95% CI	Fisher's exact probability
TT	18 (37.5)	18 (41.9)	0.83	0.36 – 1.92	**0.830**
TC	22 (45.8)	19 (44.2)	1.07	0.47 – 2.42	**1.0**
CC	8 (16.7)	6 (14.0)	1.23	0.40 – 3.84	**0.778**
Total	48 (100.0)	43 (100.0)			
Alleles frequency					
T	58 (60.0)	55 (64.0)	0.86	0.47 – 1.56	**0.649**
C	38 (40.0)	31 (36.0)	1.16	0.64 – 2.11	**0.649**

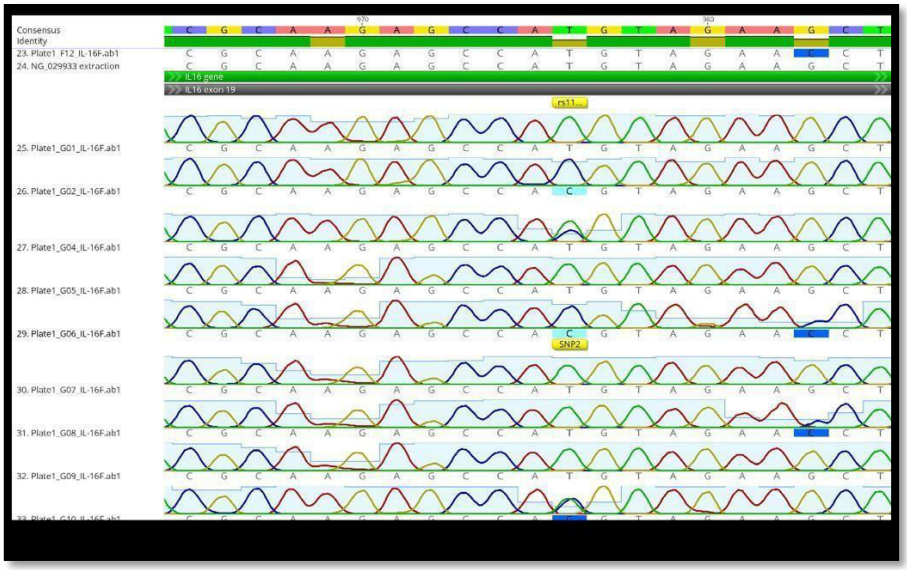

Fig. 5: The alignment of the nitrogenous bases of a part of the IL-16 gene between alopecia areata patients and a control group showing the location of rs1131445.

Table 7: IL-16 serum level distribution according to the IL-16 SNPs of thestudied groups

Genotyping of IL16 SNP rs113225	IL-16 level means ± SE (pg/ml)		Probability
	Patients group	Control group	
GG	210.0 ± 26.20 ^{A}	159.14 ± 29.65 ^{A}	0.207
GT	211.92 ± 49.75 ^{A}	190.15 ± 47.51 ^{A}	0.758
TT	345.11 ^{A}	59.56 ^{A}	-
Genotyping of IL16 SNP rs1131445			
TT	222.63 ± 33.93 ^{AB}	210.46 ± 39.28 ^{A}	0.687
TC	158.40 ± 28.65 ^{B}	126.25 ± 22.67 ^{A}	0.524
CC	345.80 ± 74.22 ^{A}	183.11 ± 112.88 ^{A}	0.061
Tukey test: The similar letters referred to non-significant differences (P > 0.05)			

nitrogenous bases for part of the IL-16 gene between the AA patients' group and the healthy control group, explaining the location of the rs113445 variation between the sequence of the nitrogenous bases that is an inversion and transition between bases by replacing the T (CAT) with another C (CAC) base resulting in a silent mutation and that encoded for the same amino acid histamine.

For IL-16 SNP rs113225, the results of IL-16 serum levels showed a non- significantly increased level of TT genotype compared to GG and GT genotypes of both the AA and control groups (Table 7). Also, there was a non-significant increased level of IL-16 in all AA groups' genotypes compared to the control group (Table 6). While the results of the IL-16 level relationship with the appeared genotypes of IL-16 SNP rs1131445 appeared that the patients with the CC genotype have the highest level compared to the TT and TC genotypes of the AA group, and there was a significant difference between the TT and CC genotypes compared to TC genotype (Table 7). In the control group, the TT genotype showed the highest level of IL-16 compared to the other genotypes, and no significant differences appeared between the control genotypes. Also, the results appeared non-significant increases in the level of IL-16 in all AA genotypes compared to the control genotypes (Table 7).

Discussion

Alopecia areata is a complex, polygenic disease with hundreds of single nucleotide polymorphisms (SNPs) presented in patients. Many of these polymorphisms have been found in the genes coding for the immune system phenotype, including the activation and function of T lymphocytes, target T regulatory cells, cytokine-producing cells, cellular expression and antigen presentation (Petukhova *et al*., 2010). Many of these genes are also associated with other autoimmune or immune diseases such as inflammatory bowel disease, multiple sclerosis, type 1 diabetes, and psoriasis (Petukhova and Christiano, 2016; Abbas *et al*, 2023).

The results of IL-16 serum level showed a non-significantly increase in the AA group compared to controls (214.56 ± 22.48 *vs.* 167.07 ± 23.59 pg/ml). The present results agree with the results reported by Tanyasiri *et al*. (2005), while disagreeing with the results of Tomaszewska *et al*. (2020), who referred to a significantly increased level of IL-16 in the AA group compared to controls.

Alopecia areata is a complex, polygenic disease with hundreds of single nucleotide polymorphisms (SNPs) present in patients. Many of these polymorphisms have been found in genomic regions involved in the immune system phenotype, including activation and function of T lymphocytes, Treg-regulated T cells, cytotoxic T cells, cellular expression and antigen presentation (Petukhova *et al*., 2010). Many of these genes are also associated with other autoimmune or immune diseases such as inflammatory bowel disease, multiple sclerosis, type 1 diabetes, and psoriasis (Petukhova and Christiano, 2016).

The current study showed polymorphisms of the IL-16 gene and its relation to alopecia areata. According to the presented results, two loci of heterogeneity were found for the IL-16 gene rs11325 and rs1131445. It is being highlighted as an important genetic marker in the pathogenesis of alopecia areata, which IL-16 rs11325 was recorded for three genotypes (GG, GT, and TT) that correspond to two alleles (*G* and *T*). while IL-16 rs1134455 showed three genotypes (TT, TC, and CC) that correspond to two alleles (*T* and *C*). Regarding the Hardy-Weinberg equilibrium (HWE), the AA and control groups' genotypes were compatible with the equation and no apparent differences between observation and expected genotype frequencies for both groups (p=0.5148, p=0.2574). The present results showed that the high OR values of GG genotype and the *G* allele for IL-16 rs11325 (1.44 and 1.31, respectively) have an important relative fraction

in the pathogenesis of alopecia areata, while the genotypes GT, TT and *T* allele were considered preventive factors for the disease at OR= 0.69, 0.89, 0.76, respectively.

Also, the findings of IL-16 rs1131445 were presented with three genotypes (TT, TC and CC) and their alleles *T* and *C*. No significant differences were recorded when comparing the frequency percentage of these genotypes and alleles between the studied groups (p=0.830, 1.0, 0.778 and 0.649, respectively). The results showed a non-significant decreased percentage of the TT and TC genotype and *T* allele in the AA group compared to the control group, while the CC genotype represented a non- significant increased percentage in the AA group compared to the control group. In addition, the low OR values of the TT genotype and *T* allele considered this genotype and allele as a preventive factor from the disease development (OR = 0.83, 0.86, respectively). While the non-significant increase in the percentage of the TC and CC genotypes and *C* allele in the AA group compared to the control group considered these genotypes and allele as the relative risk of alopecia areata development. This is consistent with the findings of Lew *et al*. (2014) in a Korean population. IL-16 gene contributes to the risk of alopecia areata developing. Hence, the polymorphisms of IL- 16 may play a role in the pathophysiology of alopecia areata or the expression of alopecia areata phenotypes (Lew *et al.,*2014). More studies are needed to clarify the role of IL-16 in the pathogenesis of alopecia areata symptoms.

The present study is the first Arabian study about the role of IL-16 serum level and genetic polymorphisms in the development of alopecia areata. So, more Arabian genetic studies are needed -including other variants- to clarify and enhance the association of these genes with the susceptibility to developing a treatment for alopecia areata. The samples size and no financial support were the main part of the limitation of the present study.

Conclusion

This study is the first study on the Arabian population and Iraq. The current findings concluded that the level of IL-16 has increased in the alopecia areata patients' group, and the GG genotype and G allele for IL-16 rs11325 and TT, TC, and *T* allele for IL-16 rs1131445 have a relative fraction in the alopecia areata patients' group. GG genotype and *T* allele for IL-16 rs11325 and TC and CC genotypes and *C* allele of IL- 16 rs1131445 are etiological factors and constitute a risk ratio for infection with alopecia areata due to the higher OR value for the studied SNPs of IL-16. Changes in the nitrogenous bases result in genetic mutations of the substitution type, which leads to a change in the type of amino acid. More genetic studies should be conducted to ensure the role of IL-16 in the development of alopecia areata.

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