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Association of Interleukin-12B Polymorphism and Serum Level of Interleukin-12 in a Sample of Iraqi Patients with Rheumatoid Arthritis

Layla Hassan Khayon, Abbas Aboud Farhan, Dunya Fareed Salloom
Department of Biology, College of Education for Pure Sciences, University of Diyala, Diyala, Iraq.
Department of Biology, College of Sciences, University of Baghdad, Baghdad, Iraq.
***Corresponding author:** layla.hassan.khayon@uodiyala.edu.iq, Mobile: +9647723220927

ABSTRACT

Background: In rheumatoid arthritis, your immune system attacks the tissue lining the joints on both sides of your body. Other parts of the body may also be affected. Unsure of the exact cause. Two separate genes termed IL12A (p35) and IL12 encode the heterodimeric cytokine known as IL12 (p40). Several different hematopoietic cell types can have several different hematopoietic cell types that can generate antigen-presenting cells (APCs), including DCs and macrophages.
Objectives: This study aimed to investigate if the interleukin IL-12B gene's common polymorphisms in an Iraqi population were associated with RA.
Material and methods: Blood samples were taken from 70 Iraqi patients with RA illnesses and 30 Iraqi controls during the periods from April 2022 to June 2022 at Baghdad Teaching Hospital and Typical Rheumatology Unit. IL-12 level was determined by ELISA, and the IL-12B gene SNP was investigated through RT-PCR.
Results: Between the sick and the healthy group, there was no statistically significant difference in the levels of IL-12**.** The allele G was more prevalent, and the genotype GG was more noticeable in patients compared to healthy people. As a result, the pattern represents a risk factor for RA (OR (95% CI, 1.55, (0.47 - 5.12), P=0.523).
Conclusion: We concluded that the IL-12B gene SNP rs3212227 GG was linked to the onset of RA, and that people carrying the G allele had a greater probability of doing so.
Keywords: Rheumatoid arthritis, IL- 12, SNP.

INTRODUCTION

The lining of the joints throughout the body is attacked by the immune system in the autoimmune disease rheumatoid arthritis (RA) [(1)]. Inflammation of the joints brought on by rheumatoid arthritis can, in extreme circumstances, lead to lifelong joint damage and disability. The lungs, heart, blood vessels, skin, and eyes are just a few of the many organs that RA may impact. Rheumatoid arthritis affects around 1 in 200 persons globally, and women are often affected 2 to 3 times more often than males. Although it can affect anyone at any age, the peak onset is between the ages of 50 and 59 [(1)]. Joint discomfort, especially in the hands and feet, and stiffness that lasts longer than 30 minutes in the morning are the earliest symptoms of RA. Joints afflicted by RA become swollen and challenging to move as the condition worsens. Times of greater inflammation (flares) are frequently followed by periods of relative improvement as the pain and swelling frequently come and go. Patients may also have flu-like symptoms during flares, including exhaustion and muscular pains [(1)].

Interleukin 12 (IL-12) family consists of the four proteins IL-12, IL-23, IL-27, and IL-35. When an antigen is presented to naïve T cells by activated antigen-presenting cells (APC), IL-12, IL-23, and IL-27 are produced, but IL-35 is a consequence of regulatory T and B cells. They achieve this by promoting the transition of immature CD4+ T cells into memory T cells and cytokine-producing T-helper subsets, which serve as a bridge between the innate and adaptive immune system (2). IL-12 cytokines govern the immune system's cellular pathways, some of which stimulate pro-inflammatory responses that defend against infection while others regulate unregulated immunological responses that cause autoimmune disorders. These cytokines also affect the choices made by differentiating lymphocytes about their cell destiny **[(3)]**.

In the rheumatology unit, there were 53786 total patients, 1039 of them had rheumatoid arthritis. 50.1% of people live in urban areas, while 49.9% do so in rural ones. Incidence was 1.60 in 2001 and 3.02 in 2011 for the same group [(4)].

MATERIALS AND METHODS

Blood samples of 70 cases with a recognized diagnosis of RA based on the information about clinical symptoms and laboratory tests that were obtained from Baghdad Teaching Hospital and Typical Rheumatology Unit. In addition, a control group included 30 apparently healthy subjects in term of non-RA; their age range was 20-67 years. Also, age demographic data were similar in the both subject groups**.**

Measurement of serum IL-12 levels

Both the RA patients and the healthy controls had their blood drawn. ELISA kits (Al-shkairate, Jordan) according to the manufacturer's instructions, were used to

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measure the concentrations of serum IL-12. The optical density was calculated at 450 nm using an automated ELISA reader.

Blood sample and DNA extraction

DNA was taken from blood samples utilizing the Gene aid, Taiwan's gSYNCTM DNA Extraction Kit.

Genotyping

The IL-12B gene SNPs, rs3212227 was genotyped in 70 patients with RA and 30 control subjects using Real-Time PCR. Probe for the SNP was purchased from Thermo Fisher Scientific (USA).

Ethics approval: The project for this study was approved from The Ethical Committee at the University of Diyala, College of Education for Pure Sciences.

Statistical Analysis

For the parametric data, the mean and standard error were calculated using IBM SPSS version 27.0, and the probability was calculated using an ANOVA table and an independent T-test. While Pearson chi-square is used to determine the probability for non-parametric data. When the probability was less than 0.05, it was considered significant. An Online Hardy-Weinberg equilibrium calculator was used to determine Fisher's exact probability, the odd ratio, and the 95% confidence interval.

RESULTS

RA disease based on IL-12 concentration

Table (1) indicated no appreciable variations in IL-12 levels between the RA patients and the control group.

Table (1): Comparison of samples studied in accordance with patient and control IL-12 level data

Groups	IL-12 (mean ± SE, Unit)	Probability
Patients group	15.64 ± 2.85	0.346 NS
Control group	10.64 ± 0.28	

Gene polymorphisms for interleukin-12B

The IL12B gene's genetic polymorphism at position (IL12B-3212227), which has three genotypes, was studied (TT, TG, and GG). In contrast to controls, there was no discernible difference between the observed and predicted genotype frequencies for IL12B-3212227 in RA patients (P 0.05). (i.e., a deviation from H-W equilibrium). The genotype or allele frequencies between RA patients and controls did not show any obvious differences (Tables 2 and 3).

Table (2): The genotypes and alleles of IL12B-3212227 were identified in RA patients and controls using counts, percentage frequencies, and Hardy-Weinberg (H-W) equilibrium

Genotypes of rs321222	Patients group No. (%)		Control group No. (%)	
	Observed	Expected	Observed	Expected
	No. (%)	No. (%)	No. (%)	No. (%)
TT	0 (0.0)	0.2 (0.3)	0 (0.0)	0.2 (0.7)
TG	8 (11.4)	7.5 (10.8)	5 (16.7)	4.6 (15.3)
GG	62 (88.6)	62.2 (88.9)	25 (83.3)	25.2 (84.0)
Total	70 (100.0)	70 (100.0)	30 (100.0)	30 (100.0)
P-HWE	0.6121		0.6185	

Table (3): Statistical analyses of connections between the genotypes or alleles of IL12B-3212227 and RA

Geno-types of rs3212 227	Patients group No. (%)	Control group No. (%)	OR (95% CI)	EF or PF	Fisher's exact probability
TT	0 (0.0)	0 (0.0)	-	-	-
TG	8 (11.4)	5 (16.7)	0.65 (0.20 – 2.13)	5.9	0.523
GG	62 (88.6)	25 (83.3)	1.55 (0.47 – 5.12)	31.4	0.523
Total	70 (100.0)	30 (100.0)			
Alleles frequencies					
T	8 (6.0)	5 (8.0)	0.67 (0.21 – 2.11)	2.8	0.536
G	132 (94)	55 (92.0)	1.50 (0.47 – 4.75)	31.4	0.536

Effect of the IL12B-3212227 SNP on IL-12 serum levels

The greatest mean of IL-12 was observed by the IL-12-3212227 TG genotype in RA patients, however, the difference from all other genotypes in patients or controls was not statistically significant (table 4).

Table (4): Effect of the IL12B-3212227 SNP on IL-12 serum levels

Genotypes of rs3212227	IL-12 mean ± SE (Unit)		P-value
	Patients group	Control group	
TT	-	-	-
TG	19.05 ± 7.98[A]	-	-
GG	15.18 ± 3.06[A]	10.64 ± 0.28	0.397
Duncan test: a non-significant difference was shown by the same letters.			

DISCUSSION

The Hardy-Weinberg equilibrium was supported by the genotype frequencies for the SNP ($P > 0.05$). An Iraqi study found that those with rheumatoid arthritis had greater levels of pro-inflammatory cytokines such as IL-12 [(5)]. In a different investigation, it was shown that asthmatic patients had lower IL-12 concentrations than the healthy group [(6)].

When compared to the control group, patients had considerably greater frequencies of the genotypes AC, CC, AC+CC, and C allele. Serum IL-12 levels in patients were significantly higher than in the control group [(7).]

The RA group and control group had substantially different frequencies of the AC+CC genotype of the IL-12B gene (rs3212227). Patients with RA and control participants had substantially different rs3212227 allele frequencies. The minor allele (C) frequency of the IL-12B gene, rs3212227 was significantly higher in RA patients than in controls [(8)]. Different IL-12B gene polymorphisms may change how the T cell-mediated immune response develops and enhances the vulnerability to various autoimmune disorders, such as RA [(9).]

Contrasted with the control group, serum IL-12 level in RA group was considerably greater. RA patients had statistically greater rates of the rs3212227 CC genotype than did the control group [(9).]

According to one study, there is no connection between the start of rheumatoid arthritis and the IL-12B polymorphism [(10)]. No significant correlation between IL-12B (rs3212227) and risk of RA was found in studies including 3436 RA patients and 4644 healthy controls. Additionally, no connection between RA and the IL-12B (rs6887695) and IL-12A (rs2243115) polymorphisms has been found [(11).]

The heterogeneity of the illness, ethnic differences in genetic variation between groups, and the small sample size may all contribute to these inconsistencies between research.

CONCLUSION

We came to the conclusion that the IL-12B gene SNP rs3212227 GG was linked to the onset of RA, and people carrying the G allele had a greater probability of doing so**.**

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