Original Article

The Relationship between −330 Interleukin-2 Gene Polymorphism and Its Plasma Levels in Patients with Alopecia Areata

Sarah Isam Al-Rubaye, Abdulsamie Hassan Alta'ee, Zena Saeed Al-Fadhily[1]

Departments of Biochemistry and [1]Internal Medicine, College of Medicine, University of Babylon, Hillah, Iraq

Abstract

Background: Alopecia areata (AA) is an autoimmune, dermatological, chronic, inflammatory disease that attacks hair follicles and causes hair loss. Hair loss usually occurs on the scalp, but it can also affect the beard, eyebrows, and other areas of the body. Interleukin-2 (IL-2) is a cytokine that contributes to the regulation of the immune system and is classified as a proinflammatory factor. IL-2 is an autocrine secretary element produced from activated T-cells, exhibiting growth factor characteristics. **Objective:** The objective of this study was to investigate the effect of the −330 IL-2 gene polymorphism (rs2069762) on plasma IL-2 levels in Iraqi patients with AA. **Materials and Methods:** In this study, 100 patients with AA and 100 ethnicity-, age-, and sex-matched healthy controls were selected. Blood samples of all individuals were collected in EDTA tubes. The restriction fragment length polymorphism–polymerase chain reaction method was applied to determine various alleles and genotypes in these individuals. Plasma concentration of IL-2 was measured in all the samples using human IL-2 kit. **Results:** The frequency of −330 G/T IL-2 genotype was higher in patients with AA compared to normal individuals. Accordingly, the plasma levels of IL-2 were significantly higher ($P < 0.0090$) in patients when compared to the control group. **Conclusion:** In case of patients with AA, the −330 G/T IL-2 genotype is associated with higher plasma levels of IL-2.

Keywords: −330 interleukin-2 polymorphism, alopecia areata, hair loss, interleukin-2

Introduction

Alopecia areata (AA) is a dermatological, chronic, inflammatory, autoimmune disease, which targets the hair follicles, causing hair loss. The hair loss usually occurs on the scalp, but can also affect the beard, eyebrows, and other areas of the body.[1] Alopecia primarily affects hair follicles, but it can also affect fingernails, causing small indentations and roughness. Most individuals experience onset of alopecia by the age of 40 years, with nearly half experiencing onset before the age of 20 years. In the United States, approximately 500,000 individuals have AA. AA tends to occur in three different patterns: focal, totalis, and universalis[2] in addition to androgenetic alopecia.[3] For patients with alopecia totalis and universalis, onset is typically before the age of 30 years. In children, the mean age of onset is between 5 and 10 years of age.[2]

Focal pattern alopecia consisting of one or multiple hairless patches appears on the scalp. Alopecia universalis consists of complete hair loss on all parts of the body, whereas alopecia totalis consists of total hair loss on the scalp. Patients with alopecia may experience periods of hair regrowth and hair loss throughout the course of the disease.

Interleukin-2 (IL-2) is classified as a pro-, anti-inflammatory factor; it is a cytokine contributed with immunological response regulation.[1] It acts as a signal molecule, which participates in the natural immune response by binding to receptors of IL-2 that are expressed by the lymphocytes which are the cells responsible for immunity. IL-2 gene is a major functional factor, which is involved in immune regulation and function.[4]

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Address for correspondence: Prof. Abdulsamie Hassan Alta'ee, Department of Biochemistry, College of Medicine, University of Babylon, Hillah, Iraq.
E-mail: abdulsamie68@gmail.com

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IL-2 is an autocrine secretary element produced by activated T-cells and has growth factor properties. IL-2 has an exciting effect on the T-cell proliferation and differentiation. IL-2 has a critical role in reservation of peripheral T-cell tolerance; it has a critical role in regulatory T-cell (Treg) equilibrium, and deterioration of Treg cells could be involved in the autoimmunity as a result of IL-2 disturbance.[1] IL-2 plays a major role during T-cell development in the thymus for the maturation of a unique category of T-cells called Tregs;[5] Treg function prevents other T-cells from identifying and reacting with the self-antigens, which may lead to autoimmunity by preventing the responding cells from producing IL-2. Considering these informations, IL-2 distinguishes between self and non-self, which is one of the immune system characteristics.[6]

Many studies have demonstrated that many of IL-2 gene polymorphisms and other genes that participate in immune system are involved in autoimmune diseases.[7] Because the impact of −330 IL-2 polymorphism on the plasma concentration or expression of IL-2 in patients with AA has not been reported, the present study aims to report the impact of the −330 IL-2 gene polymorphism (rs2069762) on the IL-2 plasma levels of Iraqi patients with AA.

Materials and Methods

Patients and control groups

One hundred distinct patients with AA were participated in the present study. These patients were selected from Baghdad Teaching Medical City and were diagnosed by a neurologist based on the McDonald criteria.[8] The mean and standard deviation age was 26.00 ± 9.47 for males and 23.68 ± 11.65 for females in the patient group, and the age range was 15–48 years.

Furthermore, one hundred ethnicity-, gender-, and age-matched healthy individuals without any personal/family history of any autoimmune diseases were selected. The mean age was 29.42 ± 12.93 for males and 31.53 ± 7.078 for females in the control group, and the age range was 15–48 years.

DNA extraction and genotyping

Five milliliters of blood sample was taken from each participant in an EDTA tube. The DNA was extracted from the blood samples using a DNA extraction kit. Then, the DNA samples were subjected to a polymerase chain reaction (PCR)–restriction fragment length polymorphism. One hundred nanograms of extracted DNA were amplified using specific primers: direct 5'-ATTCACATGTTCAGTGTAGTTCT-3' and reverse 5'-GTGATAGCTCAATTCATGC-3'. PCR conditions were 95°C for 3 min, followed by 35 cycles of 45 s at 95°C, 45 s at 56°C, 45 s at 72°C, and a final extension step of 7 min at 72°C.[9]

PCR amplification gave a band of 131 bp. After digestion with the restriction enzyme Bfa-1 (Takara), the PCR products were digested into 110 and 21 bp fragments. Two percent agarose gel electrophoresis was used. The plasma concentration of IL-2 was detected using a human IL-2 kit purchased from eBioscience (http://www.eBioscience.com). The kit has been used in accordance with the manufacturer's instructions. Finally, a comparison was made between the genotype −330 IL-2 and the plasma level of IL-2.

Statistical analysis

To investigate the effect of IL-2 −330 polymorphism and plasma IL-2 concentration, the independent Student's *t*-test was performed. The differences between the parameters determined in patients with AA and in the control group are compared, and $P < 0.05$ was considered statistically significant. Version 6 of GraphPad Prism (GraphPad Software Inc., La Jolla, California, USA) was used for Windows software.[10]

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with the patient's verbal and analytical approval before the sample was taken. According to this approval, all the samples were collected and the tests were carried out.

Results

The frequency of the T allele at the −330 IL-2 polymorphism was insignificantly higher in patients than controls (odds ratio (OR): 0.6321, confidence interval (CI): 0.2687–1.487, and $P = 0.3889$). Moreover, the G/T genotype was more frequent in patients than that in controls (36% vs. 10%, OR = 5.063, CI = 0.9491–27.00, and $P = 0.0476$) as shown in Table 1. Allelic association of (−330 G/T) IL-2 Gene Polymorphism in Promoter AA patients and healthy controls is shown in Table 2. The relationship between IL-2 and genotyping (−330G/T) IL-2 gene of polymorphism in promoter is shown in Table 3.

The patients who carried T/G genotype had higher plasma concentration of IL-2 compared to that measured in controls ($P = 0.0138$). The concentration of serum IL-2 of the

Table 1: The genotype and percentage of interleukin-2

Genotype	IL-2			*P*
	Control (Group 1, *n*=100), *n* (%)	Patients (Group 2, *n*=100), *n* (%)	OR (CI)	
G/G	30 (30)	28 (28)	5.464 (1.626-18.36)	0.0476
T/T	60 (60)	36 (36)	0.3750 (0.1116-1.260)	
G/T	10 (10)	36 (36)	5.063 (0.9491-27.00)	

IL: Interleukin, OR: Odds ratio, CI: Confidence interval

patient group was found to be significantly increased in patients with AA when compared with control groups ($P = 0.0090$), as shown in Table 4 and Figure 1.

Discussion

Because IL-2 helps maintain the immune response and plays a fundamental role in self-tolerance activity, its alteration could be implicated in the etiology of autoimmune diseases such as AA. Therefore, different genotypes of −330 IL-2 may affect plasma levels of IL-2 in patients with AA.

Table 2: Allelic association of (−330 G/T) interleukin-2 gene polymorphism in promoter alopecia areata patients and healthy control

Allele	Count (%)		OR (CI)	*P*
	Patients (Group 2)	Control (Group 1)		
G	92 (46)	70 (35)	0.6321 (0.2687-1.487)	0.3889
T	108 (54)	130 (65)		

OR: Odds ratio, CI: Confidence interval

Table 3: Relationship between interleukin-2 plasma level and genotyping (−330G/T) interleukin-2 gene of polymorphism in promoter

Genotype	IL-2 level±SD (ng/mL)		*P*
	Control	Patients	
TT	26.29±5.43	29.23±4.66	0.2090
GG	24.30±4.01	30.01±7.48	0.1548
TG	23.75±3.26	30.34±3.33	0.0138

IL: Interleukin, SD: Standard deviation

Table 4: Serum interleukin-2 and cytotoxic T-lymphocyte antigen-4 concentration in patients with alopecia areata and healthy controls

Parameters	Studied groups (mean±SD)		*P*
	Control (Group 1)	Patients (Group 2)	
IL-2 (ng/mL), *n*	25.41±4.78, 100	29.53±5.18, 100	0.0090

SD: Standard deviation, IL: Interleukin

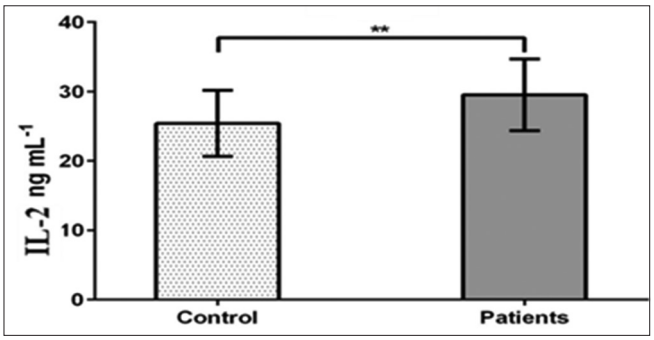

Figure 1: Serum interleukin-2 in patients with alopecia areata and healthy controls

Several lines of study have concluded that IL-2 is involved in the pathogenesis of AA. IL-2 levels have been reported to be elevated in the serum of patients with autoimmune diseases, including patients with AA.[11] John *et al.* (1998) detected the single-nucleotide polymorphism of the IL-2 −330 promoter and reported that "both −330 IL-2 G/T and T/T genotypes of the IL-2 gene had an association with susceptibility to progressive secondary progression of multiple sclerosis." They observed that the IL-2 promoter in the cell line exhibited significantly higher levels of gene expression at −330. In contrast, they found that IL-2 expression in lymphocytes increased in carriers of G/T and T/T genotypes.[7]

The present study examined the relationship between the −330 G/T genotype of the IL-2 gene and the plasma concentrations of IL-2 in Iraqi patients with AA and revealed that the genotype −330 T/G genotype was significantly more frequent in patients with AA and was related to a higher plasma concentration of IL-2 comparing to controls, suggesting that −330 G/T genotype of IL-2 gene could have an effect on increasing IL-2 plasma concentration. The study showed that plasma levels are significantly higher in patients with AA than that in healthy controls, and these levels are increased in patients with AA who carried G/T genotype, suggesting that there would be a relation between G/T genotype and plasma levels of IL-2 in patients with AA.

These results are agreed with a study that concluded that the serum levels of IL-2 were significantly elevated in patients with AA, indicate that IL-2 may play a role in the pathogenesis of AA, assume that the elevated serum levels of IL-2 may reflect the inflammatory symptoms in AA, and that control of IL-2 production may be important to the management of this disease.[11]

The present study agreed with another previous similar study by Sayad *et al.* that reported a significantly increased levels of serum IL-2 in cases of another autoimmune disease (multiple sclerosis) compared with healthy individuals.[7] The results of the present study suggest that IL-2 family cytokines might be involved in the pathogenesis of AA and that levels of IL-2 family cytokines might reflect the activity of the disease; these results support the hypothesis that IL-2 family cytokines are involved in the pathogenesis of AA.

Conclusion

A higher plasma concentration of IL-2 was observed in patients with AA with G/T genotype.

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Conflicts of interest

There are no conflicts of interest.

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