The Association between (CT60) Cytotoxic T-lymphocyte Antigen-4 Gene Polymorphism with its Plasma Levels in Alpecia Areata Patients

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

[1]College of Medicine, University of Babylon, Iraq

ABSTRACT

Alopecia areata (AA) is a dermatological disease featured by non-scarring hair loss of the scalp and/or body, with a variable and unpredictable development in the patients in whom, despite diverse efforts, its cause is not yet fully known, some signs suggest that genetic, environmental and immunological factors may cause the disease. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) said negative T cell proliferation regulator by attenuating either T-cell response post-activation or by preventing the activation of self-reactive T cells. The present study, aims to report the impact of the (CT60) CTLA-4 gene polymorphism (rs3087243) on the CTLA-4 levels in plasma samples of AA Iraqi patients.

Keywords: *Alopecia areata, Hair loss, CTLA-4, CT60 Polymorphism.*

Introduction

Alopecia areata (AA) is a dermatological disease characterized by hair loss and no scars on the head and/or body, manifested by a different and unpredictable development for patients in which, despite various experiments, the cause is not yet known, some evidence suggests for the genetic, environmental and immunological factors to cause the disease. [1]. AA is a chronic, inflammatory, autoimmune disease that attacks the hair follicles and causes hair loss. Hair loss is usually on the scalp, but it can also affect the beard, eyebrows and other areas of the body. Genes of the immune system have been implicated in AA pathogenesis [2]. The CTLA4 gene (2q34) produces a full-length CTLA4 protein, predominantly expressed in activated T cells, and a soluble CTLA4 protein, predominantly secreted by resting T cells [3]. CTLA-4 is a negative regulator of the T-cell proliferation either by reducing T-cell response post-activation or preventing the activation of self-reactive T cells [2].

Corresponding Author:
Abdulsamie Hassan Alta'ee
College of Medicine, University of Babylon, Iraq,
P. Code 51002, Hilla PO Box 473, Iraq
Email: abdulsamie68@gmail.com

CTLA-4 polymorphisms are associated with a lot of human autoimmune-diseases, including systemic lupus, Graves disease, rheumatoid arthritis and type I diabetes [4,5]. More than 100 single-nucleotide polymorphisms (SNPs) have been identified in the CTLA4 region 2q34 and several variants have been associated with autoimmune disorders [6]. However, some of these associations have not been confirmed, and some contradictory data are evident in different populations. Two recent articles show that certain genetic variants of the CTLA4 gene promote AA sensitivity. The strongest relationship was found in the change of + 49AG infection (rs231775), resulting in a threonine to alanine amino acid substitution at codon 17 in the leader peptide, and for the (CT60) dimorphism (rs3087243), which is located 236bp downstream of CTLA4 gene ([2,7]). The present study, aims to report the impact of the (CT60) gene polymorphism (rs3087243) on the CTLA-4 levels in plasma samples of AA Iraqi patients.

Patients and Control Groups

One hundred (100) distinct patients with AA were selected in this study. The patients had mean standard deviation age of (26.00 ± 9.47), for males and (23.68 ± 11.65) for females and age range of 15-48 years. These patients were selected from Baghdad teaching medical city and diagnosed by neurologist based on the McDonald criteria [8].

Besides, one hundred (100) ethnically, age, and sex matched healthy individuals with no personal or family history of autoimmune diseases were selected. Control group had mean age of (29.42 ± 12.93) for males and (31.53 ± 7.078) for females and age range of 15-48 years. All controls were informed of the research and gave their full written consent.

DNA Extraction and Genotyping: Five mL of blood sample was collected from each individual in EDTA tube and plasma isolated. DNA was extracted from peripheral blood samples by DNA extraction kit. Then DNA sample was subjected to restriction fragment length polymorphism PCR (RFLP). 100 nanograms of extracted DNA were amplified using specific primers: CT60-forward 5' -ATCTGTGGTGGTCGTT TTCC-3' and CT60-reverse 5' -CCATGACAACTGTAAT GCCTGT-3' primers The PCR conditions were 95 °C for 3 min followed by 35 cycles of 45s at 95 °C, 45s at 60 °C, 45s at 72 °C, and a final prolongation step of 7 min at 72 °C.

The PCR amplification yielded a band of 382 bp. After digestion by HpyCh4IV restriction enzyme (New England Biolabs), the PCR products were digested 252 and 130 bp fragments. 2% agarose gel electrophoresis was used. Plasma levels of CTLA-4 were detected using a Human CTLA-4 ELISA kit purchased from eBioscience company (http://www.eBioscience.com). The kit was used according to its manufacturer's instruction. Finally, the comparison between CT60 genotype and CTLA-4 plasma level was done.

Statistical Analysis: To examine the effect of CTLA-4 polymorphism and its plasma levels, independent Student's *t*-test was performed. Differences between the parameters measured in patients and control group were compared, and *P* value was <0.05 considered significant. Gravepad Prism version 6 (Gravepad Software Inc., La Jolla, CA) for windows software was utilized [9].

Results and Discusion

The frequency of the G allele at the (CT60) polymorphism was insignificantly higher in patients than controls (OR: 0.8851 CI: 0.3825 to 2.048, and *P* = 0.8323). Moreover, the A/G genotype was more frequent in patients than in control (68% versus 50%, OR: 2.125, CI: 0.6308 to 7.159, and *P* = 0.3772) as shown in Table 1. Allelic Association of (CT60) CTLA-4 Gene Polymorphism in Promoter AA patients and Healthy Control was shown in Table 2. Relation between CTLA-4 and genotyping of (CT60) CTLA-4 gene polymorphism in promoter were shown in Table 3.

Table 1: The Genotype and Percentage of CTLA-4

Genotype	CTLA-4			P value
	Control (G1, N = 100)	Patients (G2, N = 100)	OR/CI	
G/G	30 (30%)	24 (24%)	0.7368 (0.1957 to 2.775)	0.3772
A/A	20 (20%)	8 (8%)	0.3478 (0.05671 to 2.133)	
G/A	50 (50%)	68 (68%)	2.125 (0.6308 to 7.159)	

Table 2: Allelic Association of (CT60) CTLA-4 Gene Polymorphism in Promoter AA Patients and Healthy Control

	Patients Group (G2)		Controls Group (G1)		OR	(CI)	P-value
Allele	Count	Proportion	Count	Proportion			
G	116	58%	110	55%	0.8851	(0.3825 to 2.048)	0.8323
A	84	42%	90	45%			

The patients who carried A/G genotype had higher plasma levels of CTLA-4 compared to that measured in controls (*P*= 0.0312). Figure 1 showed that CTLA-4 levels were lower in patients than in controls.

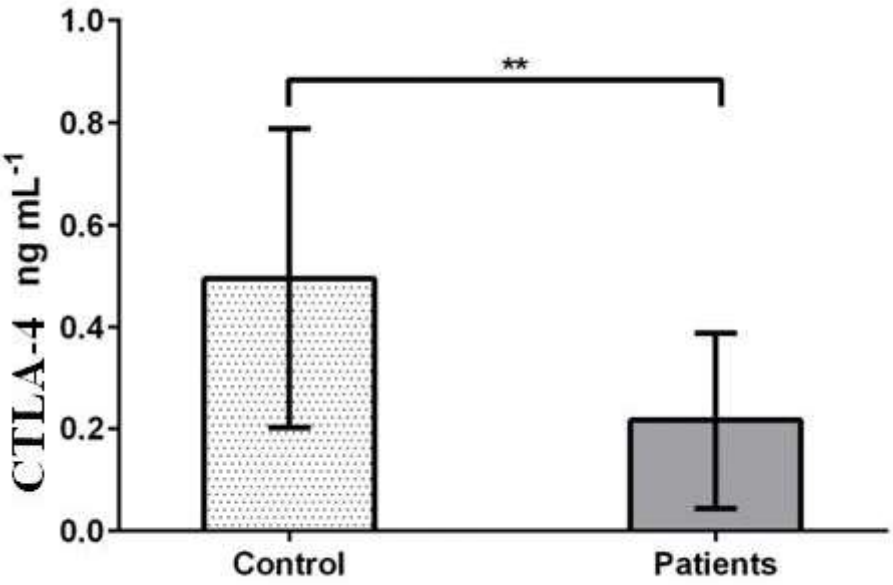

Figure 1: Serum Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) in AA Patient and Healthy Control Groups

Table 3: Relation between CTLA-4 and Genotyping of (CT60) CTLA-4 Gene Polymorphism in Promoter

Genotype	Control Group (G1) (CTLA-4 level ± SD) ng/mL	Patients Group (G2) (CTLA-4 level ± SD) ng/mL	P value
AA	0.47 ± 0.31	0.25 ± 0.18	0.1252
GG	0.45 ± 0.23	0.14 ± 0.10	0.0167
AG	0.59 ± 0.35	0.07 ± 0.028	0.0312

CT60 G/A genotype of CTLA-4 gene may have an effect on decreasing CTLA-4 plasma level in general population (e.g., control group). Since carriers of G/A genotype in the AA patients group have significantly lower CTLA-4 levels in comparison to healthy controls, it seems that maybe there are additional mechanisms that affect or control the relation of G/A genotypes and CTLA-4 levels in AA patients.

In this study, we investigated the association of the G/A genotype at the CT60 position of the CTLA-4 gene with the plasma levels of CTLA-4 in Iraqi AA patients. We observed that this effect is significantly higher in AA patients than in healthy controls. It shows there would be relation between this SNP and level of CTLA-4 in AA patients. It seems that studies with large sample size are required to bring about more authentic results [10]. CTLA4 is an important negative regulator of T-cell activation, whose polymorphisms have been reported to be implicated in the genetic susceptibility of several autoimmune diseases, recently also Alopecia Areata 11. In this study, we evaluated the association between CT60 SNPs in CTLA4 gene using 100 patients and 100 healthy individuals from Iraq. Using a case–control approach, we found that AA was significantly more frequent in patients carrying the (CT60) G allele (G/G and A/G genotypes) than in A/A homozygotes, while A/A genotype seemed to have a protective effect. Our findings are agreed with those obtained in the German study performed by John et al [9]. in which a significantly decrease of (CT60) A variant was present in AA cases than in controls. Even the fact that the association is specific for A/G G/G genotypes agrees with the dominant effect of the G allele that we speculated. Furthermore, a specific haplotype of the CTLA4 gene, carrying the (CT60) A variant, resulted to be negatively associated to AA evolution. A previous study suggested that CTLA4 molecular pathway plays a major role in the AA pathogenesis, and might also manifested that the two genetic risk factors, CTLA4 and HLA, act autonomously of each other. The study continued saying "Regarding +49AG CTLA-4 SNP, very similar frequencies for either genotype or allele were observed in patients and controls. These data, however, did not replicate the

association with the +49G CTLA-4 variant reported in German people and genetic heterogeneity among European populations, SNP interactions might explain this result" [2,11]. We cannot exclude the possibility that other CTLA-4 genetic variants present are involved in the autoimmune etiology of the disease. In conclusion, the carriage of the (CT60) G allele participate, even if moderately, as a disease risk factor of AA, which is encourage the importance of CTLA4 as an autoimmune susceptible locus. Since CTLA4 molecule has a major role in the measurement of immune response and tolerance, it is probably that some particular CTLA4 polymorphisms could be causes the differences in the individual capability to have autoimmune diseases, altering the function of the protein itself. Notice that, the (CT60) SNP is located near the 30 un-translated region of CTLA4 gene, and the association of several diseases with the G variant has been explained as the result of a reduced CTLA4 expression with a subsequent reduced inhibitory function [12]. Further investigations is required to investigate the effect of the (CT60) G allele on the possible binding of some major genetic factors, for example the microRNA regulatory molecules and the concentration and function of the CTLA4 translated protein with a view to find out new molecular mechanisms implicated in the AA development. Moreover, the research for disease preparing genetic variants in other immunological pathways and co-stimulatory molecules must be investigated to give clear vision into the AA pathogenesis.

Conclusion

Lower plasma levels of CTLA-4 in AA patients appeared in those who carried G/A genotype.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicine, University of Babylon, Iraq and all experiments were carried out in accordance with approved guidelines.

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