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Lack of Association between Interleukin-1 β Gene Polymorphisms and Alopecia Areata; A Case-Control Study

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Abstract

Background: Alopecia Areata (AA) is an autoimmune disease characterized by T-cell infiltrates and cytokine production around anagen hair follicles. The aetiology of AA includes genetic susceptibility, environmental and immunological factors.

Objective: To study the possible association of IL-1 β-C-511T and C+3953T Single Nucleotide Polymorphism (SNP) with AA.

Methods: This case-control study was conducted on 100 AA patients and 100 controls. Three ml venous blood was withdrawn from every participant for genotyping of IL-1B-511 C/T, and IL-1B +3954 C/T SNPs using PCR-RFLP technique.

Results: The studied genotype distribution and allele frequencies showed no significant difference between AA patients and healthy controls, apart from IL-1 β+3953 C allele that was found more frequently than T allele among cases and controls.

Conclusion: IL-1 β C-511T and C+3953T SNPs have no etiological role in our studied Egyptian AA patients.

Key Words: *Alopecia areata – Genetic predisposition – Interleukin-1.*

Introduction

ALOPECIA Areata (AA) is the most frequent cause of hair loss induced by inflammation [1]. It is an autoimmune disease characterized by T-cell infiltration and cytokine production around anagen hair [2].

The possession of specific alleles of polymorphic genes can influence the susceptibility or severity of a number of disorders [3]. One of the several loci reported to have significant association imbalance with AA is located on chromosome 2q13-21. This includes the Interleukin (IL)-1 cluster genes, IL-1 α, IL-1 β, and IL-1RA. Variations in these genes can modulate the efficacy of IL-1 signaling predisposing to disease [4].

Biallelic polymorphisms at positions –511 (rs16944, C>T) in the promoter region and +3954 (rs1143634, C>T) in exon 5 have likely useful implication in modulating IL-1 protein production and are linked to the development of some diseases [5]. The IL-1 β gene polymorphisms are of specific importance in AA, since pro-inflammatory cytokines are shown to have association in the inhibition of human hair growth. Besides, higher IL-1β mRNA was described in scalp biopsies of patients with alopecia totalis [6].

Aim of the study:

Our aim was to investigate the role of the IL-1β C-511T and C+3953T Single Nucleotide Polymorphism (SNP) in the pathogenesis of AA.

Patients and Methods

This hospital based case-control study was conducted on 100 AA patients and 100 age and sex matched apparently healthy controls. The study period ranged between May 2014 and November 2015. All subjects were Egyptians and were consecutively collected from the Outpatient Clinic of Dermatology Department of Kasr Al-Aini Faculty of Medicine, Cairo University after the approval of the Dermatology Research Ethical Committee (DermaREC) of the Faculty of Medicine, Cairo University and the ethical committee of the National Research Center, Egypt.

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Exclusion criteria included patients with any known autoimmune diseases. One hundred apparently healthy age and sex matched controls coming to the clinic with their relatives were included in the present study.

Genotyping ofIL-1B-511 C/T and IL-1B +3954 C/T SNPs:

Three ml venous blood was withdrawn under complete aseptic conditions from every participant in sterile Ethylene Diamine Tetra-Acetic Acid (EDTA) vacutainer tubes. Genomic DNA extraction from peripheral blood leucocytes was done using AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences, USA) according to manufacturer's instructions. The concentration and purity of the recovered DNA were assessed by spectrophotometry, and the samples were stored in elution buffer at –20°C until use.

Detection of IL-1B -511 C/T SNP was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique [5]. The primer sets used were Forward: 5' -TGG CAT TGA TCT GGT TCA TC-3' and Reverse: 5'-GTT TAG GAA TCT TCC CAC TT-3'. The PCR conditions were initial denaturation step at 95ºC for 5min. followed by 30 cycles of denaturation at 95ºC for 1min., annealing at 55ºC for 1min. and extension at 72ºC for 1min. followed by a final extension step of 5min. at 72ºC. PCR product was incubated with 2.5U of Ava1 restriction enzyme (FermentasTM-Lithuania) at 37ºC overnight. The resulting fragments of 190bp+114bp were considered as allele-1 whereas allele-2 remained uncut resulting in a single 304bp fragment.

For IL-1B+3954C/T genotyping by PCR-RFLP assay, the following primer pair was used Forward: 5'-AGG TGT CCT CCA AGA AAT CAA A- 3' and Reverse: 5' -GCT TTT TTG CTG TGA GTC CCG-3'. The optimized PCR conducted was initial denaturation at 95ºC for 2min. followed by 30 cycles of denaturation at 95ºC for 30sec., annealing at 60ºC for 30sec. and extension at 72ºC for 30sec. followed by a final extension step of 5min. at 72ºC. PCR product was incubated with 10U of Taq I restriction enzyme (FermentasTM-Lithuania) at 37ºC overnight. The restriction pattern was observed on 2% agarose gel under UV after staining with ethidium bromide. C allele was identified by 2 fragments of 108+86bp, while T allele was an unrestricted band of 194bp [5].

For quality control, genotyping of the studied SNPs was repeated for 30 samples randomly with respect to case/control status. The results of genotyping were interpreted blindly by two different observers and were found to be 100% concordant.

Statistical methods:

Data were statistically described in terms of mean ± standard deviation (±SD), median and range, or frequencies (number of cases) and percentages when appropriate. Odds Ratio (OR) with its 95% Confidence Interval (CI) was calculated for the different genotypes as well as for the allelic frequency of each gene between cases and controls. Comparison of numerical variables between the study groups was done using student *t*-test for independent samples in comparing cases versus controls and Kruskal Wallis test when comparing the different genotypes. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. *p*-values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

Results

This case-control study included 100 AA cases and 100 age and sex matched healthy controls (*p*=0.091 and 1 respectively).

The cases group included 32 females (32%) and 68 males (68%) with mean age of 22.9 years. 70% (n=70) of cases had no family history of AA while 30% (n=30) had a positive family history of AA. 86% (n=86) of cases had no family history of other autoimmune diseases and 14% (n=14) had a positive family history of other autoimmune diseases. The control group included 32 females (32%) and 68 males (68%) with mean age of 24.9 years.

IL-1β-511, and IL-1β+3953 gene polymorphisms among cases and controls:

The distribution of IL-1β-511, and IL-1β+3953 gene polymorphisms and allelic frequencies in cases and controls have been demonstrated in (Table 1). The studied genotype distribution and allele frequencies showed no significant difference between patients and controls, apart from IL-1β +3953 C allele that was found more frequently than T allele among cases and controls. Moreover, the AA cases had 64.2% increase in the odds to carry the IL-1β+3953 T allele and 39.1% lower odds to carry the IL-1β+3953 C allele than the healthy controls (Table 1).

Studying the association between different gene polymorphisms with the presence of family history of AA or other autoimmune diseases among cases revealed no significant association (Table 2).

Table (1): Genotype distribution and allele frequencies among the study groups.

	Cases n (%) (n=100)	Controls n (%) (n=100)	OR	95%CI	*p*-value
IL-1β-511 genotypes:					
CC	36 (36%)	28 (28%)	1.000	0.497-2.011	1 (ref.)
CT	43 (43%)	47 (47%)	0.712	0.374-1.355	0.3
TT	21 (21%)	25 (25%)	0.653	0.305-1.400	0.273
CT/TT	64 (64%)	72 (72%)	0.691	0.380-1.257	0.225
T allele	85 (42.5%)	97 (48.5%)	0.785	0.529-1.164	0.228
C allele	115 (57.5%)	103 (51.5%)	1.274	0.859-1.890	
IL-1β+3953 genotypes:					
CC	54 (54%)	67 (67%)	1.000	0.602-1.660	1 (ref.)
CT	39 (39%)	30 (30%)	1.613	0.889-2.927	0.115
TT	7 (7%)	3 (3%)	2.895	0.714-11.731	0.224
CT/TT	46 (46%)	33 (33%)	1.730	0.975-3.068	0.06
T allele	53 (26.5%)	36 (18%)	1.642	1.018-2.650	0.041*
C allele	147 (73.5%)	164 (82%)	0.609	0.377-0.982	

N: Number.
* : *p*-value <0.05 is statistically significant.

Table (2): Association between different gene polymorphisms and the presence of family history of alopecia areata or other autoimmune diseases among cases.

Genotype	Family history of AA		*p*-value	Family history of other autoimmune diseases		*p*-value
	Present	Absent		Present	Absent	
IL-1ß-511:						
CC (n=36)	10 (27.8%)	26 (72.2%)	0.34	6 (16.7%)	30 (83.3%)	0.75
CT (n=43)	11 (25.6%)	32 (74.4%)		6 (14%)	37 (86%)	
TT (n=21)	9 (42.9%)	12 (51.7%)		2 (9.5%)	19 (90.5%)	
IL-1ß+ 3953:						
CC (n=54)	18 (33.3%)	36 (66.7%)	0.55	10 (18.5%)	44 (81.5%)	0.09
CT (n=39)	11 (28.2%)	28 (71.8%)		2 (5.1%)	37 (94.9%)	
TT (n=7)	1 (14.3%)	6 (85.7%)		2 (28.6%)	5 (71.4%)	

N: Number.
* : *p*-value <0.05 is statistically significant.

Discussion

The importance of studying genetic susceptibility of AA shoots from it being a relatively frequently encountered disorder affecting 0.1%-0.2% of the population worldwide [7], in addition to its interference with the quality of life [8]. We aimed to investigate the role of the IL-1 β SNP C-51 1T and C+3953T in the pathogenesis of AA. Our current observations add to the evidence suggesting genetic heterogeneity in AA.

This study did not show any significant difference between patients and controls regarding studied gene polymorphisms. This was in agreement with Tazi-Ahnini et al., [9] who found no significant difference in the allelic distribution of IL-1 β-511 SNP between their British patients and controls. IL-1 β-511 CC genotype and C allele have been previously linked with higher risk of other diseases such as rheumatoid arthritis [10].

In this case-control study, the non-significant association between IL-1 β+3953 SNP and AA was also in agreement with the findings of Alfahdi and Nanda [11]. Yet, our AA cases had higher risk to carry the IL-1 β+3953 T allele and lower risk to carry the IL-1 β+3953 C allele than healthy controls.

IL-1 β+3953 T allele has been previously linked with higher risk of inflammatory diseases such as chronic periodontitis [12].

Interleukin-1 is a key regulator of the host response and a major modulator of extracellular matrix catabolism and bone resorption **[12]**. IL-1β is a proinflammatory cytokine mainly produced by blood monocytes and tissue macrophages and has been implicated in mediating both acute and chronic inflammation **[13]**. IL-1β can promote the movement of inflammatory cells from the blood to inflamed tissues, regulate the extracellular matrix and induce other cytokines **[14]**. Polymorphisms in IL-1β gene have been reported to affect the level of gene expression **[15]**.

The discrepancy between this study and other studies regarding the association of AA with IL-11β SNP C-51 1T and C+3953T could be explained by the different genetic background of the studied population, the different methodology and the different sample size. Moreover, the association of a gene polymorphism with altered protein production, that can play a role in the pathogenesis of certain diseases, may occur due to linkage with another factor directly affecting gene expression **[16]**. More studies with large population and with analysis of overall defined clinical data are in need.

As a conclusion, there was no overall association between IL-11 β C-51 1T and C+3953T SNPs and AA in our studied cases.

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الملخص العربى

داء الثعلبة هو أحد أمراض المناعة الذاتية التى تتميز بوجود الخلية (تى) وأنتاج السيتوكينات حول بصيلات الشعر اللتى فى طور التنامى.

من ضمن مسببات داء الثعلبة: القابلية الوراثية والبيئية والعوامل المناعية.

الهدف من هذا البحث هو دراسة احتمال وجود ارتباط بين داء الثعلبة وتعدد الأشكال النووية المنفرد للأنترلوكين ١ بيتا C+3953T (IL-1β) و C-511T.

أجريت هذه الدراسة على ١٠٠ حالة تعانى من داء الثعلبة و١٠٠ حالة من أشخاص أصحاء.

تم سحب ثلاثة مل من الدم الوريدى من كل مشارك لدراسة تعدد الأشكال النووية المنفرد للأنترلوكين ١ بيتا C+3953T (IL-1β) و C-511T باستخدام تقنية PCR-RFLP.

\النتائج: دراسة التوزيع الوراثى وترددات الأليل لم تظهر أى اختلاف كبير بين المرضى والأشخاص الأصحاء. ولكن وجد أن ١β+3953C أليل متواجد بنسبة عالية فى المرضى والاشخاص الاصحاء.

\الخلاصة: تعدد الأشكال النووية المنفرد للأنترلوكين ١ بيتا C+3953T (IL-1β) و C–511T ليس له أى دور كمسبب لداء الثعلبة فى المرضى المصريين فى هذه الدراسة.