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Association of Human Beta – Defensin 1 Gene Polymorphisms with Alopecia Areata Patients

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Abstract

Alopecia areata (AA) is a common disease with an incidence of 2-3% among the dermatoses and 0.1% in the population at large. This disorder occurs in both sexes, at all ages, and is characterized by the sudden appearance of areas of hair loss on the scalp and other hair bearing areas. Various factors, including immunologic and endocrine abnormalities, genetic factors, infections, and psychological/ psychiatric disturbances, have been claimed to play a role in its etiopathogenesis. The aim of this study is to investigate the association between DEFB1 polymorphism and AA pathogenesis. This study was conducted on fifty patients with alopecia areata (Group A) and fifty age and sex matched apparently healthy subjects as a control group (Group B). Patients were selected from the Dermatology outpatient clinic of Benha University Hospital. Linear regression analysis was conducted for prediction of more severe AA; using age, gender, family history, AID, previous episodes, previous treatment, and rs1800972 genotypes as covariates. Rs1800972 (CG+GG) were considered as predictor of more severe AA ($p<0.001$). Our study results support the associations of susceptibility with AA among patients with the DEFB1 rs1800972 genotypes. It appears that DEFB1 gene polymorphisms may modulate AA risk. So, CG, GG, genotypes and G allele were considered as predictor of AA susceptibility and severity.

Keywords: AA, AID, DEFB1 and CG.

1. Introduction

Alopecia areata (AA) is an autoimmune disease that presents as non-scaring hair loss. It is a common condition affecting 0.1-0.2% of population and can occur at any age without sex predilection. Although in many cases it can be a self-limiting condition, hair loss can cause emotional and psychosocial distress**.**

Alopecia areata is characterized by small round or oval bald patches. The underlying skin is unscarred and looks superficially normal. Alopecia areata most often affects the scalp and beard, but may occur on any part of the body with hair. It can affect entire scalp (alopecia totalis) or cause loss of all body hair (alopecia universalis) **[1]**. It is a T cell-mediated autoimmune disease characterized histologically, by infiltrating T cells surrounding the hair follicle bulb [2].

The aetiology of AA remains elusive. Autoimmunity is believed to play an important role in disease pathogenesis; as AA is also associated with other autoimmune disease. Both T-cell subsets; CD4+ and CD8+ T cells have a role in the induction of pathologic findings. The intrafollicular T-cell infiltrate is pre- dominantly composed of CD8+T cells. In contrast, the perifollicular infiltrate is composed primarily of CD4+T cells [3].

The role of immunological response in AA mediated by some involved cytokines and chemokines (4). There is also a direct role for IFN-γ–producing $CD8^+$ cytotoxic T-cells in progression of AA corresponding to a T- helper 1 response. In addition, T helper- 17 response has already been acknowledged to play an important role in AA pathogenesis. Th-17 cells produce IL-17, IL-23 and IL-6; and Tregs synthesize transforming growth factor (TGF) -β and IL-10 [5].

Th 17 response is charecterized by elicitation of anti-microbial peptides (AMP) through (IL) 17A, (IL) 17F and IL22 signalling, leading to localized inflammation**.**

An excess of AMPs such as human beta defensin (HBD)-1 may contribute to a local inflammation; for HBD-1, this is due to its ability to chemoattract neutrophils , immature dendritic cells and T cells directly through chemokine receptor (CCR6) signalling and indirectly by HBD-3 induction [6].

Certain polymorphisms in DEFB1, the gene encoding for HBD-1, may affect its transcription rate and the expression of HBD-1 protein [7]. In particular polymorphisms in the 5 untranslated regions (UTR) of DEFB1 exert this effect by altering a putative transcription factor binding site for the nuclear factor-jB p105 subunit [8].

Considering autoimmunity, HBD-1 and its gene polymorphisms have been evaluated in psoriasis, type 1 diabetes (T1D), oral lichen planus (OLP),inflammatory bowel disease (IBD), and systemic and cutaneous lupus erythematosus**.** However, to date, there are no similar studies regarding AA susceptibility.

2. Subjects and methods

Type of the study: A prospective case-control study.

2.1 Subjects

This study was conducted on Fifty patients with alopecia areata (Group A) and Fifty age and sex matched apparently healthy subjects as a control

group (Group B). Patients were selected from the Dermatology outpatient clinic of Benha University Hospital.

2.2 Methods

Sample Collection, DNA Extraction, Gene amplification using polymerase chain reaction and 4-Detection of PCR Amplification products using Gel Electrophoresis and Ultra- Violet Light Trans illumination.

2.2.1 Sample collection

A venous blood sample (2 ml) were withdrawn from each subject and collected into sterile ethylene diaminetetra acetate "EDTA" (vacutainer) tube and were used for DNA extraction. DNA was extracted from fresh samples and stored at -20 °C till time of assay for determination of MTHFR A1298C >T gene polymorphism by using real time Polymerase chain reaction (RT- PCR) technique .

2.2.2 DNA Extraction

The blood samples were used for DNA extraction using the Gene JET Whole Blood Genomic DNA purification Mini Kit (Thermo Fisher Scientific, Germany) according to the manufacturer's protocol.

2.2.3 Gene amplification using polymerase chain reaction

Enzymatic amplification was performed by PCR using 1-star^{TM} Taq DNA polymerase enzyme (intro biotechnology) and Bold Thermal Cycler (ABI Applied Biosystem).

3. Results

The rs1800972 SNP had C and G alleles. C is the ancestral. It is located on the short arm of chromosome 8 within DEFB1 gene.

This sample of individuals was selected randomly from population in Qaliobeya Governorate in Egypt. Applying Hardy Weinberg equation revealed that rs1800972 genotypes in control as well as in cases, groups were in HW equilibrium .

Taking rs1800972 CC as the reference genotype and C as the reference allele; CG, GG, CG+GG genotypes and G allele showed significantly higher frequency in AA when compared to control group (p=0.047, 0.004, 0.004, <0.001 respectively) with risk to develop AA (OR=1.864, 5.312, 2.326, 2.289 respectively) Table (1).

No significant differences were found in clinical data between rs1800972 genotypes in all studied AA cases.

SALT score increased significantly in CC, CG, and GG respectively, in CG+GG when compared to CC genotypes.

Logistic regression analysis was conducted for prediction of AA development using age, gender, family history, AID, rs1800972 genotypes as covariates. Rs1800972 (CG+GG) were independent risk predictors for AA development.

Linear regression analysis was conducted for prediction of more severe AA; using age, gender, family history, AID, previous episodes, previous treatment, rs1800972 genotypes as covariates. Rs1800972 (CG+GG) were considered as predictor of more severe AA.

Table (1) Comparison of clinical data between rs1800972 genotypes in all studied AA cases.

		CC N=15	CG N=16	GG N=9	CG+GG N=25	P[1]	P[2]
Previous episodes		4 26.7%	5 31.3	3 33.3	8 32%	0.933 F	0.722[F]
Disease duration (months)		1.3 ±0.4	1.2 ±0.3	1.6 ±0.4	1.3 ±0.3	0.743 A	0.952[T]
Number of lesions	**Single**	9 60.0%	10 62.5%	5 55.6%	15 60.0%	0.944 F	1.000[C]
	Multiple	6 40.0%	6 37.5%	4 44.4%	10 40.0%		
Site	**Scalp**	14 93.3%	15 93.8%	8 88.9%	23 92.0%	0.896 F	0.877[F]
Nail pitting		1 6.7%	1 6.3%	0 0.0%	1 4.0%	0..736 F	0.708[F]
Previous treatment	**Negative**	12 80.0%	15 93.8%	6 66.7%	21 84.0%	0.274 F	0.676[F]
	IL steroids	1 6.7%	1 6.3%	2 22.2%	3 12.0%		
	Topical steroids	2 13.3%	0 0.0%	1 11.1%	1 4.0%		

P1, comparison between CC, CG, GG; p2, comparison between CG+GG versus CC; SD, standard deviation; A, ANOVA; T, student t test; F, Fisher exact test; C, chi square test.

4. Discussion

Alopecia areata (AA) is a recurrent, nonscarring hair loss, affecting any hair-bearing area. Its incidence is 0,1-1%. It is considered to be a T-cell-mediated autoimmunity occurring in genetically predisposed individuals [10].

The aetiology of AA remains elusive. Autoimmunity is believed to play an important role in disease pathogenesis. Supporting this theory is the fact that activated CD4 and CD8 T lymphocytes have been found in perifollicular and intrafollicular infiltrates of affected individuals"anagen hair follicles" [3]. A genome-wide association study demonstrated a genetic predisposition to AA [11].

There is little information regarding the role of HBD-1 /DEFB1 in alopecia areata .In present study , The role of DEFB1 5 UTR polymorphisms in AA is evaluated based on their previously reported association with other autoimmune diseases as psoriasis (PSO), type 1 diabetes (T1D), oral lichen planus (OLP) and systemic and cutaneous lupus erythematosus (SLE and CLE, respectively) [12].

Certain polymorphisms in DEFB1, the gene encoding for HBD-1, may affect its transcription rate and the expression of HBD-1 protein [7]. In particular polymorphisms in the 5 untranslated region (UTR) of DEFB1 exert this effect by altering a putative transcription factor binding site for the nuclear factor-jB p105 subunit [8].

In current study, the rs1800972 SNP had C and G alleles. Taking rs1800972 CC as the reference genotype and C as the reference allele; CG, GG, CG+GG genotypes and G allele showed significantly higher frequency in AA when compared to control group with risk to develop AA.

These findings are similar to previous reports in SLE [13]. But different from reports on T1D, in which the allele and genotype appeared to have a protective effect [14]. These results could be attributable to an increase in the transcription rate of DEFB1 mRNA due to the presence of the -44 G allele, increasing constitutive HBD-1 production .This could also be relevant for SLE and TID ,as observed previously [7]. Thus, our findings support the autoimmune hypothesis for AA.

The role of immunological response in AA is mediated by some involved cytokines and chemokines [4]. There is also a direct role for IFN-γ–producing $CD8^+$ cytotoxic T-cells in progression of AA corresponding to a T- helper 1 response. In addition, T helper- 17 responses has already been acknowledged to play an important role in AA pathogenesis. Th-17 cells produce IL-17, IL-23 and IL-6; and Tregs synthesize transforming growth factor (TGF)-β and IL-10 [5].Th 17 response is charecterized by elicitation of anti-microbial peptides (AMP) through (IL) 17A, (IL) 17F and IL22 signalling ,leading to localized inflammation.

An excess of AMPs such as human beta defensin (HBD)-1, encoded by DEFB1 gene, may contribute to a local inflammation. for HBD-1, this is due to its ability to chemoattract neutrophils , immature dendritic cells and T cells directly through chemokine receptor (CCR6) signalling and indirectly by HBD-3 induction [6].

Considering the chemo attractant properties of HBD-1 for immature dendritic cells and memory T cells, which in the presence of ‘danger’ signals (e.g. oxidative stress, high levels of IL-6, IL-8 and heat shock protein 70) would promote initial autoantigen presentation, intensification of inflammation, induction of cell apoptosis and reduction of immune tolerance [15].

In current study, no significant differences were found in clinical data as previous episodes of AA, disease duration, number of lesions, site and nail pitting between rs1800972 genotypes in all studied AA cases.

In current study, SALT score increased significantly in CC, CG, GG respectively and in CG+GG when compared to CC genotypes ($p<0.001$). Also, when linear regression analysis was conducted for prediction of more severe AA; using age, gender, family history, AID, previous episodes, and rs1800972 genotypes as covariates. Rs1800972 (CG+GG) were considered as predictor of more severe AA ($p<0.001$). So, rs1800972 CG, GG, genotypes, G allele was considered as predictor of AA susceptibility and severity.

Logistic regression analysis was conducted for prediction of AA development using age, gender, family history, AID, rs1800972 genotypes as covariates. Rs1800972 (CG+GG) were independent risk predictors for AA development ($p=0.004$).

In agreement with our study, Ochoa-Ramírez et al(16)found GGG was more frequent in patients with vitiligo, being associated with a 1.66-fold increased risk for developing the disorder ($P = 0.01$) .

5. Conclusion

Our study results support the associations of susceptibility with AA among patients with the DEFB1 rs1800972 genotypes. It appears that DEFB1 gene polymorphisms may modulate AA risk. So, CG, GG, genotypes and G allele were considered as predictor of AA susceptibility and severity.

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