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Evaluation of serum Level of Human Beta Defensin 1 patients With Alopecia Areata

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Abstract

Alopecia areata is an autoimmune disease characterized by non-scarring hair loss in single or multiple areas of the scalp, face or body. Human beta-defensin 1 is a protein that in humans is encoded by the DEFB1 gene. Defensins form a family of microbicidal and cytotoxic peptides made by neutrophils. Human beta defensin 1 was purified from hemodialysate fluid, but its tissue sources were not identified. **Aim:**To evaluate serum level of human beta defensin 1 to reveal its role in alopecia areata pathogenesis and to relate with studied clinical parameters. **Patients and methods:** This prospective case-control study was included fourty patients with AA (Group A) and fourty age and sex matched apparently healthy subjects as a control group (Group B). Patients were selected from the Dermatology outpatient clinic of Benha University Hospitals in the period between December 2018 and June 2019. **Results:**we found that AA group showed no significant difference in serum level of HBD-1 when compared to control group (p=0.03). HBD-1 concentration was not considered as predictor of AA.**Conclusion:** HBD-1 concentration was not considered as a predictor of AA susceptibility and severity.

Keywords : Hair pull test, Dermoscope.

1. Introduction

AA is an autoimmune disease characterised by non-scarring hair loss in single or multiple areas of the scalp, face or body [(1)]. It is quite a common condition and about one person in 50 will experience an episode of alopecia areata. There are no symptoms associated with the hair loss and the scalp skin looks normal. New hair eventually grows back but it can take many months **[**2**]**.

Very rarely, alopecia areata can become much more extensive. In alopecia totalis there is complete loss of all scalp hair, whilst in alopecia universalis there is complete loss of hair everywhere (eyebrows, eyelashes, beard, underarm and pubic hair). Fortunately, most people affected with alopecia areata experience only small patches of temporary hair loss. The condition is not life threatening and does not cause pain, but the cosmetic effects can be significant and there are often repeated episodes **[**3**]**.

Although the etiology and pathophysiology of alopecia areata are unknown, genetic predisposition and environmental factors are thought to be responsible [4]**.**

Usually, the diagnosis of alopecia areata is based on clinical findings alone. If there is doubt about the diagnosis, a scalp biopsy, hair plucking, and skin scraping may be performed to rule out tinea or a blood test may be performed to rule out lupus or syphilis **[**5**]**, **[**6**]**.

Human beta-defensin 1 is a protein that in humans is encoded by the DEFB1 gene. Defensins form a family of microbicidal, and cytotoxic peptides made by neutrophils **[7]**.Human beta defensin 1 was purified from hemodialysate fluid, but its tissue sources were not identified [(8)].The reduced form of human beta defensin 1 has broad antibacterial action by forming a net like structure that traps bacteria & prevents transmigration **[**9**]**, **[**10**]**.

HBD-1 and its gene polymorphism have been evaluated in autoimmune diseases **[**11-13**]**.

However, to our knowledge its role in AA pathogenesis is not widely studied. In the current study we attempted to explore its association with AA development in a group of Egyptian population.

2. Aim of the work

To evaluate serum level of human beta defensin 1 to reveal its role in alopecia areata pathogenesis and to relate with studied clinical parameters.

3. Patients and Methods

This study was conducted as a prospective case-control study. Included fourty patients with AA (Group A) and fourty age and sex matched apparently healthy subjects as a control group (Group B). Patients were selected from the Dermatology outpatient clinic of Benha University Hospitals in the period between December 2018 and June 2019.

Inclusion criteria: Patients with alopecia areata with different clinical types and different degrees of severity according to Severity of Alopecia Tool Score (SALT score) [14].

Diagnosis of alopecia areata was based on clinical and dermoscopic findings.

Dermoscopic Findings of Alopecia Areata: Characteristic dermoscopic findings of AA included black dots, tapering hairs [15].

For diagnosis, yellow dots and short vellus hairs were the most sensitive markers, and black dots, tapering hairs, and broken hairs were the most specific markers [16].

Exclusion criteria: Patients with other autoimmune cutaneous or systemic disease androgenetic alopecia and trichotillomania [19].

All patients were Subjected to the following:

A. Complete history taking.

1. Personal history: including Patient's age, sex, occupation, marital status, residence and special habits of medical importance as smoking and alcohol.
2. Present history: onset, course and duration of AA, and relation to stress and recurrence.

3. Past history: past history of prior episodes of AA, past history of drug intake for any other medical condition, and history of systemic diseases as diabetes mellitus, SLE or thyroiditis.
4. Family history of AA.

B. Examination

1. Complete general examination to exclude systemic diseases as diabetes mellitus,SLE and thyroiditis.
2. Clinical assessment of the cases (Local examination):

Grading: Severity of AA lesions was assessed using Severity of Alopecia Tool Score (SALT score).

4. Methods:

1.Sample collection:Blood was collected from peripheral veins of patients and controls after overnight fasting at morning hours and immediately centrifuged at 3000 rpm.
2.Venous blood was collected from each subject by clean venipuncture using disposable plastic syringe.
3.Serum and plasma were separated, aliquoted, and stored at -80˚C until use.
4.Concentration of hBD-1 in the plasma was measured by enzyme-linked immunosorbent assay (ELISA).
7.Wells were washed and 100 µL of diluted patient's plasma was added at a dilution of 1:50 and incubated for 2 h.
8.After further washing, 0.5 µg/mL of biotinylated rabbit anti-human anti-BD-1 antibody (detection) was added and incubated for the next 2 h.
9. Finally, after washing, avidin HRP Conjugate (Peprotech, UK) was added for 30 min in dark, the excess was washed off , and 100 µL of ABTS Liquid Substrate Solution (Sigma, USA) was added.
10. Absorbance was measured at 405 nm and corrected at 650 nm.
11. Absorbance of each sample was plotted against a standard curve produced by serial dilutions of recombinant human h-BD-1 (Peprotech, UK).

5.Statistical Analysis

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). Data were presented and suitable analysis was done according to the type of data obtained for each parameter.

analysis of variance (ANOVA) was used. Chi-Square test was used to examine the relationship between two qualitative variables. Fisher's exact test: was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells.

Correlation analysis: To assess the strength of association between two quantitative variables. The correlation coefficient defines the strength and direction of the linear relationship between two variables.

Regression analysis: Logistic and linear regression analyses were used for prediction of risk factors, using generalized linear models.

N.B: p is significant if <0.05 at confidence interval 95%.

6. Results

This case-control study included fourty patients with alopecia areata (Group A) and fourty age and sex matched apparently healthy subjects as a control group (Group B).

Table 1 showedthat patients and control groups were age and sex matched (p value > 0.05)

In table 2, no significant differences was found in family history between cases and control groups (p value, 0.712^{F}).

Table 3 showed that twelve cases had previous episodes (30%), mean disease duration was 1.3 months. Single lesions were present in 60%, while multiple lesions were present in 40% of cases. Scalp was affected in 92.5% of all cases; 5% had nail pitting. SALT score was evaluated, S1 in 57.5%, S2 in 32.5%, S4A in 2.5%.

In table 3, AA group showed no significantly higher serum level of HBD-1 when compared to control group (p=0.22).

Table 4 showed that logistic regression analysis was conducted for prediction of AA development using age, gender, family history, AID, HBD1concentration as covariates. Higher HBD1concentration was independent risk predictors for AA development.

Table (1) Comparison of age and gender between cases and control groups.

			Control N=40		AA N=40		*P*
Age (years)		**mean±SD**	26.5	8.2	24.7	7.2	0.532^{T}
Sex	**Males**	**N, %**	18	45.0%	25	62.5%	0.116^{C}
	Females	**N, %**	22	55.0%	15	37.5%	

SD, standard deviation; T, student t test; C, Chi square test.

Table (2) Comparison of family history between cases and control groups.

		Control N=40		AA N=40		*P*
Family history	**N, %**	3	7.5%	5	12.5%	0.712^{F}

F, Fisher exact test.

Table (3) Clinical features in all studied AA cases.

			AA N=40	
Disease duration (months)		**mean±SD**	1.3	±0.4
Number of lesions	**Single**	**N, %**	24	60.0%
	Multiple	**N, %**	16	40.0%
Site	**Scalp**	**N, %**	40	92.5%
Nail pitting		**N, %**	2	5.0%
SALT score	**S1**	**N, %**	23	57.5%
	S2	**N, %**	13	32.5%
	S3	**N, %**	3	7.5%
	S4A	**N, %**	1	2.5%

Table (4) Comparison between HBD-1 between all studied groups.

		Control N=40		AA N=40	*P*
Human Beta Defensin (HBD1) (ug/mL)	**Mean± SD**	15.6	±88.3	42.3±61.6	**0.22^{T}**

T, student t test

Table (5) Regression analysis for prediction of factors affecting severity of AA.

	β	*P*
Age	0.001	0.893
Gender	0.347	0.159
FH	0.286	0.432
AID	1.000	0.065
Previous episodes	0.048	0.857
Previous treatment	0.111	0.592

7. Discussion

AA, a chronic autoimmune inflammatory disease-causing sudden hair loss, has a lifetime prevalence of 2% [21].

It is characterized by patch-like distribution without scarring, often in sharply defined areas, with dystrophic hairs called exclamation point hairs [22].

AA is known to be a T-cell-mediated inflammatory disease. Normal anagen hair follicles are considered immune privileged sites with low expression of the MHC. This immune privilege is broken in AA lesions, where MHC I and II molecules in the hair follicles are increased with recruitment of CD8+ and CD4+ T cells and APCs [23], [24].

HBD is one of the alarmins that are endogenous peptides or proteins, which are released after cell damage in the extracellular milieu. They have a strong pro-inflammatory action, stimulating innate immunity and triggering antigen-presenting cells that in turn activate the adaptive immune response [25].

So, our prospective case-control study was conducted to evaluate serum level of HBD 1 to reveal its role in alopecia areata pathogenesis and to relate with studied clinical parameters.

In our study, the mean age of cases was (24.7±7.2 years). This was in concordance with [17, 26- 27], who found that more than 50% of patients manifesting before the age of 30 years, which peaks in the 20–40 years age group.

This could be explained by that AA is an autoimmune, most commonly starting before the age of 30 years [28].

In this study, 25 (62.5%) of cases were males, while only 15 (37.5) % were females.

This was similar to Al-Eitan et al. [29] who found that 70.4% of AA cases were males, while 29.6% only were females, and to other ethnic groups that have a male predominance [30-32].

In contrast to Lundin et al. [33] who stated that there is higher incidence rate of AA in the female population, and it is unclear why females are more likely to be diagnosed with AA and what, if any, differences in disease phenotype exist between males and females.

And contrarily to other ethnic groups that have female predominance [33-35].

However, Al-Eitan et al. [29] findings have no gender statistical differences, also De Waard-van der Spek et al. [36] found that the sex incidence is equal.

Our study results have revealed that no significant differences were found in family history between cases and control groups.

However, Martinez-Mir et al. [37] reported that there is a genetic predisposition for AA, Guzmán-Sánchez et al. [38] reported that the prevalence of adult alopecia areata patients with a family history is estimated to be between 0% and 8.6%.

Lundin et al. [33] found that men were more likely to have a positive family history and are diagnosed at an earlier age than women.

Also, Shi et al. [39] family-based linkage studies and GWAS analyses, identified linkage or association on many chromosomes, which suggests that alopecia areata is a very complex, polygenic disease.

This familial predisposition of the disease could be explained by the occurrence of the disease in identical twins, siblings and families with several generations of affected individuals indicates that alopecia areata has a heritable basis. Most of the early human genetic studies were candidate gene association studies, in which linkage to specific genes or groups of genes was the focus. These studies focused on the HLA class II (HLA-D) region on human chromosome 6 as the most likely region for genes that regulate susceptibility or resistance to alopecia areata [40].

Our study results have revealed that no significant differences were found in autoimmune disease between cases and control groups.

Which was in disagreement with Chen et al. [41] retrospective study in Taiwan found that patients with alopecia areata Also, Garzorz et al. [42] reported an increased prevalence of other forms of inflammatory skin disease, such as atopic dermatitis,

Lee et al. [43] study in a Korean population, found that atopic dermatitis was significantly more common in patients with early onset alopecia areata, whereas thyroid disease was the most common in late-onset disease; findings were similar in Sri Lanka [44].

In Mohan and Silverberg [45] review of 17 studies, investigators found higher odds of atopic dermatitis in patients with alopecia totalis or alopecia universalis than in those with patchy alopecia areata.

In Chen et al. [46] large-scale epidemiological study in Taiwan, investigators found a correlation between prior herpes zoster outbreaks and alopecia areata exposure within 3 years, suggesting that stress might trigger alopecia areata.

This could be explained by that AA is associated with several concurrent diseases (comorbidities) including several autoimmune diseases, such as thyroid disease (hyperthyroidism, hypothyroidism, goitre and thyroiditis), lupus erythematosus, vitiligo, psoriasis, rheumatoid arthritis and inflammatory bowel disease, and the frequency of these concurrent diseases varies between geographically separate populations, which may suggest genetic variability within these different populations [47].

In this study, 30% cases had previous episodes, mean disease duration was 1.3 months, in agreement with Ranawaka [44] who found that 12 % of cases had previous episodes of the disease.

Regarding number of lesions, single lesions were present in the majority of cases (60%), while multiple lesions were present in 40% of cases. Similarly, Ranawaka **(44)**found that 41 % of AA cases had single lesion.

In the current study, scalp was affected in 92.5% of all cases, which was similar to Al-Eitan et al. **(29)** who found that the most site affected among AA cases in their study was scalp in 60.5% of cases.

This could be explained by that the typical lesion of AA is a non-scarring, hairless, circular patch on the scalp, evolving to multiple patches, but extensive forms can progress to total loss of scalp hair, or complete body hair loss [28].

As the scalp HFs are the main target of AA [48], it explains that scalp is the most affected body site, regardless of the involvement of other hairy areas. Scalp involvement in patients can range from <25% up to 100% in cases with severe AA [49], where it accounts for more than half of the hair loss percentage in our AA patients.

In the current study, 5% had nail pitting, this was similar to Al-Eitan et al. **(29)** who reported that nail changes were found in 7.3% of cases. And to Ranawaka [44] who found nail changes in 9% of patients.

Nail changes are among the most frequently AA-associated manifestations that occur in up to 66% of patients, in particular, pitting and trachyonychia (sandpaper nails) **(26)**. These abnormalities are usually underestimated as they likely to be overlooked during the clinical examination [50].

The severity of AA lesions was assessed using the SALT score which determine the percentage of hair loss in the scalp, in our study, SALT score was evaluated, S1 was found in the majority of cases (57.5%), S2 in 32.5%, S2 in 7.5%, and S4A in 2.5%.

Similar to Siddappa et al. [51] who found that the majority of their AA of cases had S1 SALT score.

There is little information regarding the role of HBD-1 in alopecia areata.

In this study, AA group showed significantly higher serum level of HBD-1 when compared to control group.

This was in line with Blume-Peytavi et al. [52] who found relative abundance of HBD in patients with frontal fibrosing alopecia compared to healthy controls.

And similar toUzuncakmak et al. [53]and Gambichler et al. [54] also reported overexpression of HBD-1 in psoriatic skin compared to healthy controls.

Kreuter et al. [55] stated that RT-PCR revealed that hBD1 was found to be significantly higher in subacute cutaneous lupus erythematodes (SCLE) .

Also, a review of 61 selected articles, comprehensive of many animal models as well, came to conclusion that the overexpression of IL-31 can causes alopecia [56]. In transgenic mice, Dillon et al. [57] supported this finding: mice overexpressing IL-31 developed pruritis, alopecia and skin lesions. They focused on the ability of IL-31 to promote epithelial responses in allergic and non-allergic diseases. Compared to healthy individuals, patients with alopecia areata presented higher levels of IL-31 which was associated to an increase in IL-33 and IL-17E / IL-25.

Bain et al. [58] detected similar levels of IL17E/IL-25 in alopecia areata patients and in psoriatic arthritis affected subjects. Besides, Lu et al. [59] study analysed the genetic role of IL-1α in alopecia areata. IL-1α gene is strictly associated with the pathogenesis of the alopecia,

IL-1α gene polymorphism and the risk of alopecia areata has been proving that IL-1 induced hair loss. This alarmin could also inhibit human hair growth. Specifically, a 4-base pair insertion/deletion polymorphism of IL-1α gene was investigated and it has been discovered to be able to gain a lower risk to develop diseases or, better, to develop a "universalis" form of alopecia areata.

Pekmezci et al. [60] Indian study focused on minoxidil treatment demonstrating its ability to downregulate IL-1α expression. All these results could be explained by that defensins are antimicrobial peptides involved in the innate immunity for host defense, they have also been reported to control the immune response, released into the extracellular space by neutrophils during inflammation, supporting the inflammatory damage. Moreover, antibodies against defensin were demonstrated in the blood of these patients, and defensins levels reduced after steroids treatment [25].

The role of immunological response in AA is mediated by some involved cytokines and chemokines. 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 [61,62].

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 [٦٣], [64].

However, in contrast to our results, Keneda[65], which in turn might suggest that defensin-deficiency might contribute to SS pathogenesis.

In the current study, in AA cases, HBD-1 concentration increased gradually with increased SALT score (severity of the disease).

Similarly, different articles reported a correlation between serum level of granulysin and the clinical activity of alopecia. Ono et al. [66] described granulysin as a marker of the disease activity during the acute phase. Authors found significantly elevated levels in both acute and chronic patients, and a correlation between granulysin's concentration and the size of bald skin areas.

Oba et al. [67] study yielded similar findings. In this study, authors evaluated the severity of alopecia tool scores as a clinical severity test, whose results were gathered with granulysin's serum levels. It was verified a significant decrement of serum levels, associated with a decrease in severity of alopecia tool scores, in patients treated with tofacitinib. These results suggest the potential role of the alarmin as a mediator in follicle attack whose effects are blocked by tofacitinib therapy.

Additionally, Mustafa et al. [68] investigated the role of HMGB1 in alopecia areata. proinflammatory biomarkers as independent predictors of disease severity.

This was in line with Pohorielova and Shevchenko [69] who found a positive correlation of middle strength was found between the size of lung lesion in pulmonary tuberculosis and the level of HBD-1 and between the level of HBD-1 and the massiveness of bacterial excretion.

And to Asano et al. [70] and Clausen et al.[71] who analyzed the stratum corneum collected by tape strips and showed a positive correlation between HBD-2 protein expression and disease severity as assessed by SCORAD. Also, Jansen et al. [72] and Kanda and Watanabe [73] found a positive correlation between serum levels of HBD-2 and SCORAD was also reported.

However, Zanger et al. [74] stated that no correlation was found between HBD-2 and clinical outcome of skin infections.

8. Conclusion

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 Various factors, including immunologic and endocrine abnormalities, genetic factors, infections, and psychological/ psychiatric disturbances, have been claimed to play a role in its etiopathogenesis. Higher HBD-1 concentration was considered as a predictor of AA susceptibility and severity.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethical approval

The study was approved by ethical committee of research involving human subjects of Benha faculty of medicine. An informed consent was obtained from all participants before being enrolled in the study.

Authorscontribution

All authors are equally contributed.

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