The influence of osteopontin on the pathogenesis of alopecia areata and its association with disease severity

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Background: Alopecia Areata (AA) is an autoimmune disease affecting hair follicles. Although many details are well specified in the pathogenesis of the disease, there exist certain aspects which require more investigations. Given the fact that the increase in Th-1 immunity is the essential part of the pathogenesis, the incrimination of osteopontin, as a Th-1 cytokine, is considered appropriate in the pathogenesis of the disease. The aim of the present research was to evaluate the role of osteopontin in alopecia areata and its correlation with disease pattern and severity.

Methods: This case-control study consisted of 45 patients with alopecia areata and 45 healthy individuals. The level of osteopontin was measured through blood sampling and ELISA method.

Results: The mean plasma level of osteopontin was significantly lower in patients with alopecia areata than controls ($P<0.05$)

Conclusions: The plasma level of osteopontin in patients with alopecia areata is lower than healthy controls. Moreover, there is no significant relationship between the plasma level of osteopontin and disease severity. The clinical manifestations of alopecia areata might be a sign of the altered protective effects of osteopontin. Needless to say, more investigation is required to clarify the correlation between alopecia areata and osteopontin.

Keywords: osteopontin, alopecia areata, disease activity

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INTRODUCTION

Alopecia Areata (AA) is an autoimmune disease which affects hair follicles. The clinical presentation is patchy hair loss within normal hair-bearing skin which is non-cicatricial. The clinical variants are patchy or discoid alopecia, alopecia totalis (complete loss of scalp hair), alopecia universalis (complete loss of scalp and body hair), and ophiasis (hair loss of temporal and occipital) [1]. The lifetime incidence of AA has been reported to be 2% worldwide, which is similar in both genders [2].

The pathogenesis of AA is yet to be fully understood; however, as is so far revealed, the disruption of immune privilege in the hair follicles and immunoinhibitory signaling due to an unknown antigen (certain investigators believe in *melanocyte* as the auto antigen), result in the upregulation of antigen presenting cells, presenting antigens to immune cells, mainly CD8+ T lymphocytes [3]. Furthermore, Th1 cytokine plays a crucial role in the pathogenesis of AA by producing interferon gamma (IFN-γ) and interleukin 1 (IL-1), documented due to the high levels of these mediators in the sera and tissues of patients with AA [4].

Osteopontin (OPN), also known as Eta-1 (Early T Lymphocyte Activation-1), is a phosphorylated acidic glycoprotein that has numerous activities including cell-mediated immunity, cell survival, and inflammation. It is produced by various cells such as lymphocytes, macrophages and keratinocytes [5,6], and is the key mediator for the

function of type-1 immunity. Cell-mediated (type-1) immunity is a crucial pathway to protecting against most intracellular pathogens; with the increase in the function of this pathway, on the other hand, autoimmune destruction may take place in many organs.

It is to be noted that Eta-1 is generated by activated T cells, which is the first critical event to potentiate macrophages to produce inflammatory cytokines, eventuating in the up-regulation of type-1 cytokines, hence OPN as a Th1 cytokine.

Despite the distinguished effect of osteopontin in many inflammatory diseases such as psoriasis, and its correlation with disease outcome, few studies have investigated the role of OPN in AA [7-9].

The objective of the present study was to assess the role of osteopontin in alopecia areata, and its association with disease pattern and severity.

MATERIALS AND METHODS

The present case-control study (from June 2016 to April 2017) was comprised of 45 patients, clinically and pathologically diagnosed with alopecia areata who referred to dermatology clinic as a case group, and 45 healthy individuals as controls .

Two groups were matched regarding age and gender.

Patients younger than 65 years who completed the informed consent were entered into our investigation.

The exclusion criteria were all inflammatory conditions affect the level of plasma osteopontin including thyroid disease, diabetes mellitus, inflammatory bowel disease, bone disorders, sepsis or other active infections, or neurologic diseases such as multiple sclerosis and Alzheimer's disease, pleural effusion or pneumonia, cancers such as colon, breast, prostate and lung, or pregnancy and lactation.

By having the cases and controls answer a set of questions, we collected the data regarding age, gender, disease duration, pattern and severity of disease, disease activity, family history of alopecia areata, body mass index (BMI), severity of daily anxiety and stress, smoking and alcohol consumption. The level of anxiety and stress was evaluated by Anxiety Control Questionnaire evaluated by an expert psychiatrist.

To specify disease severity, patients were divided into three groups according to the number of patches or site of involvement: mild (defined as ≤ 3 hair-loss patches or hair loss limited to eyebrows or eyelashes), moderate (defined as >3 hair-loss patches) and severe (defined as totalis or universalis alopecia).

The disease activity was assessed in physical examination by positive hair pull test and observation of exclamation point hairs.

The blood samples were taken from all cases and controls and stored at -80° C in bottles containing anti-proteases, until the time of measurement. Measuring osteopontin level was done by ELISA kit on EDTA plasma samples that was precoated by a monoclonal antibody specific for OPN onto a microplate to bind OPN in the samples.

The data are presented as mean±standard deviation (SD) and number (percentage). The employed statistical tests were chi-square for qualitative variables, and T-test and Pearson correlation test for quantitative variables. The analyses were done through the use of SPSS version 20. P-values less than 0.05 were considered as significant. The study was reviewed and approved by the Ethics Committee of Shahid Beheshti University of Medical sciences, Tehran, Iran. IRCT registration number was IRCT2016050127707N1, August 16, 2016.

RESULTS

Out of the 90 participants, half were cases and half were controls. The former group included 17 females (37.8%) and 28 males (62.3%), with a mean age of 29.76±6.68 years .The control group consisted of 24 females (52.2%) and 22 males (47.8%), with a mean age of 29.11±7.46 years. (All demographic data are shown in Table 1)

The mean duration of the disease was more than 2 years in 55.6% of the patients.

The pattern and severity of alopecia in the cases were determined by one specialist.

As noted above, the severity of the disease was evaluated by dividing the patients into three categories of mild, moderate and severe. In this way, 20% had mild, 40% had moderate and 40% had severe forms of the disease.

The hair-loss patterns were also of discoid (patchy hair-loss), totalis (complete scalp hair-loss), and universalis (loss of all body hairs) forms, where

Table 1. Demographic data of enrolled cases and controls in the study.

Variable	Case (N=45)	Control (N=45)
Age (year)	29.76±6.68	29.11±7.46
Gender		
Male	28 (62.3%)	22 (47.8%)
Female	17 (37.8%)	24 (52.2%)
BMI		
Normal	32 (71.1%)	20 (45.7%)
Abnormal	13 (28.9%)	25 (54.3%)
Family history of alopecia areata		
Yes	17 (37.8%)	19 (41.3%)
No	28 (62.2%)	27 (58.7%)
Daily anxiety and stress		
Yes	45 (100%)	24 (52.2%)
No	0	22 (47.8%)
History of Smoking		
Yes	17 (37.8%)	18 (39.1%)
No	28 (62.2%)	28 (60.9%)
History of alcohol consumption		
Yes	11 (24.4%)	14 (30.4%)
No	34 (75.6%)	32 (69.6%)
Disease duration		
> 2 years	25 (55.6%)	-
< 2 years	20 (44.4%)	-
Disease severity		
Mild	9 (20%)	-
Moderate	18 (40%)	-
Severe	18 (40%)	-
Alopecia pattern		
Discoid	27 (60%)	-
Totalis	18 (40%)	-
Lesion activity*		
Active	43 (95.5%)	-
Inactive	2 (4.5%)	-
Osteopontin level (µmol/L)	18.78±22.03	56.53±27.01

*Disease activity evaluated by positive hair pull test

60% had discoid pattern, 40% were of totalis pattern, and only one patient was affected with universalis pattern.

Physical examination by positive hair pull test revealed that almost all patients (95.5%) had active diseases.

The mean plasma level of osteopontin was 18.78±22.03 µmol/L in cases, and 56.53±27.01 µmol/L in controls, which was significantly higher (P<0.05, Table 1).

Also, there was no correlation between age and plasma levels of osteopontin in either groups (P>0.05) (Table 2). Furthermore, no significant differences were observed between the mean plasma level of osteopontin and gender, family history of alopecia areata, history of smoking and alcohol consumption and BMI (Table 3). On the other hand, abnormal BMI was noticed in 28.9% of patients and 54.3% of healthy individuals. A positive family history of alopecia areata was detected in 37.8% of the cases and 41.3% of the controls. As shown in Table 1, the consumption of alcohol and smoking in both groups had similar frequencies.

Table 2. Correlation of age and plasma levels of osteopontin.

	Pearson Correlation	*P*-Value
Age*Osteopontin (cases)	-0.236	0.119
Age*Osteopontin (controls)	0.186	0.216

Table 3. Mean osteopontin level according to demographic characteristics.

Variable	Osteopontin Level	*P*-value
Gender		
Male	34.97±31.86	0.33
Female	41.83±29.97	
BMI		
Normal	32.57±30.06	0.05
Abnormal	45.24±31.21	
Family history		
Positive	42.09±32.59	0.295
Negative	35.09±29.92	
History of stress		
Yes	30.96±30.44	<0.001
No	59.43±21.9	
Smoking		
Yes	44.21±34.55	0.143
No	33.89±28.19	
Alcohol consumption		
Yes	39.52±30.36	0.755
No	37.23±31.47	

However, the mean plasma level of osteopontin in individuals with high levels of daily anxiety and stress was significantly lower than those without a history of stress (30.96±30.44 vs. 59.43±21.9, respectively, P>0.05). Of interest, all patients complained of severe daily anxiety and stress.

DISCUSSION

Alopecia areata is an autoimmune disease which affects hair follicles [1]. The pathogenesis is mediated by the dysregulation of immune system, specifically, increasing the T-helper-1 immunity [5].

As noted previously, several studies have revealed the role of osteopontin as a Th-1 cytokine, participating in various inflammatory regulations. Ashkar et al. demonstrated that OPN up-regulates

interleukin-12 and suppresses interleukin-10 activity, making it an early component of type 1 immunity. Also, considering that the pathogenesis of AA is the result of an increase in the immunologic activity of Th1 cells, the role of OPN is implicated.

Ganzetti et al. evaluated the role of osteopontin in alopecia areata prior to and following treatment by topical immunotherapy with 2, 3-diphenilcyclopropenone (DPCP). They showed that osteopontin plasma levels in patients with alopecia areata were higher than in healthy controls; patients achieving complete recovery after DPCP treatment, however, did not show a statistically significant reduction in OPN plasma levels [6].

In another study by Rateb et al., the tissue levels of osteopontin were measured in patients with alopecia areata via polymerase-chain reaction (PCR) and immunohistochemistry (IHC). It was revealed that the tissue level of OPN was significantly higher in patients than in controls. In other words, OPN is associated with alopecia areata occurrence and pathogenesis, but has relationship with the duration and severity of the disease [13].

In contrast to Ganzetti et al., we showed that the plasma level of OPN was *lower* in patients than in healthy controls.

Considering such contradictory results, the exact role of osteopontin in hair remains a field of interest. There is also certain evidence against the high level of osteopontin in alopecia areata.

Denhardt et al. evaluated the role of osteopontin in cellular signaling and toxicant injury [5]. They showed that osteopontin has the ability to inhibit the inflammatory mediator-induced expression of nitric oxide synthetase gene and the production of nitric oxide. Th1-type cytokines play a critical role in the pathogenesis of AA; on the other hand, Th2 lymphocyte-like response inhibits the tissue-damaging effects of Th1 lymphocytes. These findings suggest that nitric oxide possibly induces a change from Th1 to Th2 lymphocytes in the immune response.

Moreover, reduced levels of calcitonin gene-related peptide in lesions have been proposed to be effective on the pathogenesis of alopecia areata, nitric oxide increase, and the release of calcitonin gene-related peptide and its effects on vasorelaxation [10].

In another remarkable study by Sonoda et al., the dermal papilla cells of growing follicles expressed a high level of nexin-1 mRNA, encoding a potent inhibitor of serine proteases such as thrombin. The cessation of nexin-1 expression at the end of the growing phase might allow for the protease activation of osteopontin, thereby augmenting the attachment of dermal papilla cells to osteopontin and facilitating the aggregation of dermal papilla cells [11]. Furthermore, osteopontin might protect dermal papilla from the cytotoxic effects of nitric oxide, which could be produced by macrophages during catagen at the time of cell destruction and poor blood supply [12].

Of note, the different results of this study might demonstrated the negative effects associated with the absence or reduction of osteopontin, as a protective factor in dermal papilla and hair follicles, in patients affected with alopecia areata.

We further showed there is no correlation between the level of osteopontin with disease duration, severity and pattern. Interestingly, the mean plasma level of osteopontin in individuals with high levels of daily anxiety and stress was significantly lower than those with no history of stress, which might be due to the negative effect of anxiety and stress on the function and serum level of osteopontin, as patients with alopecia areata experience more anxiety and stress due to their conditions and cosmetics.

CONCLUSION

The plasma level of osteopontin is lower in patients with alopecia areata in comparison with healthy controls. Moreover, there was no significant relation between OPN levels and severity of alopecia areata.

Based on these results, particular important features could be noted. Although these substances have not been definitively proven, they could be useful for further evaluation as a scientific hypothesis. As mentioned previously, osteopontin has a protective role in the dermal papilla of the hair follicles. Any alternation in the function of osteopontin results in inflammatory processes. However, reduction in osteopontin is expected in patients with inflammatory autoimmune alopecia areata.

Ultimately, the clinical manifestations of alopecia areata might be a sign of the altered protective effects of osteopontin.

Certainly, more investigation is needed to clarify the correlation between alopecia areata and osteopontin.

Conflict of Interest: None declared.

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