Abstract

Context: Studying the link between prolactin and autoimmunity has gained much ground over the past years. Its role played in alopecia areata (AA) is not clear yet, as previous reports yielded controversial results. Aims: This study aimed to measure the serum level of prolactin and to detect the expression of its receptor in AA, in an attempt to highlight its possible role in the pathogenesis of this disease. Subjects and Methods: A case-control study of 30 AA patients and 20 controls from outpatient clinic were undertaken. Every patient was subjected to history taking and clinical examination to determine the severity of alopecia tool (SALT) score. Blood samples were taken from patients and controls to determine the serum prolactin level. Scalp biopsies were obtained from the lesional skin of patients and normal skin of controls for assessment of the prolactin receptor. Statistical Analysis: Depending upon the type of data, t-test, analysis of variance test, Chi-square, receiver operator characteristic curve were undertaken. Results: On comparing the serum prolactin level between patients and controls, no significant difference was found, while the mean tissue level of prolactin receptor was significantly higher in patients than in controls. In patients, a significant positive correlation was found between the prolactin receptor and the SALT score. Conclusions: Prolactin plays a role in AA, and this role is probably through the prolactin receptors rather than the serum prolactin level.

Key Words: Alopecia areata, cell-mediated immunity, immunology

Introduction

Alopecia areata (AA) is a form of nonscarring hair loss affecting anagen hair follicles. Its exact cause is not known, but the current body of evidence supports an autoimmune origin and strong genetic contribution, further modified by unknown environmental influences.[1] Prolactin is a pituitary hormone that is known to modulate immune responses.[2] It is generated and secreted by the lactotroph cells of the anterior pituitary gland,[3,4] acts systemically as a hormone, and locally as a cytokine.[5] Prolactin and prolactin receptor (PRLR) expression has now been demonstrated in several cutaneous cell populations, including keratinocytes, fibroblasts, sweat glands, sebaceous glands, and human scalp hair follicles.[2] It is involved in the activation and differentiation of thymic epithelial cells, thymocytes, lymphocytes, and macrophages. It also operates as part of a neuroendocrine–immune network by stimulating the release of specific cytokines.[6] It is one of the major hormonal signals that are immediately upregulated on psychoemotional and physical stress.[7]

Studying the link between prolactin and autoimmunity has gained much ground over the past years, with prolactin being investigated in several autoimmune diseases as systemic lupus erythematosus, systemic sclerosis, psoriasis vulgaris, Sjogren’s disease, and AA.[8] The role played by prolactin in AA is not clear yet, as previous reports yielded controversial results,[9-12] and one study showed no change in its level following therapy.[12] Hence, the current study was conducted to measure...
the serum level of prolactin, as well as to detect the expression of its receptor in AA patients, in an attempt to verify their possible role in the pathogenesis of this disease.

**Subjects and Methods**

This case–control study was carried out on 30 patients with AA and 20 healthy volunteers serving as controls, recruited from Kasr Al Ainy Dermatology Outpatient Clinic. The study was approved by the Dermatology Research Ethical Committee (Derma REC) of the Faculty of Medicine, Cairo University. A written informed consent was signed by each patient and control. Inclusion criteria included new cases of AA or recurrent cases who did not receive any treatment at least 2 months before the study. Patients <18 year old and those with current/history of any dermatological and/or other autoimmune disorders were excluded from this study. Pregnant and lactating females as well as patients on medications known to affect prolactin level were also excluded.

Every patient was subjected to history taking including personal data, history of the present illness, and clinical examination to determine the extent of AA using the severity of alopecia tool (SALT) score. This is a global severity score created by the combination of extent and density of scalp hair loss. It determines the amount of terminal hair loss in each of the four scalp's views then adding these together with a full score of 100%.

A 3 ml blood sample was withdrawn from all individuals to determine the serum prolactin level. A 4 mm punch scalp biopsy was obtained from the lesional skin of the patients and from the normal skin “in the occipital area” of controls for assessment of the prolactin receptor.

**Detection of serum prolactin levels and expression of its receptor**

The prolactin level was measured in serum sample of each individual by using ELISA kit which based on the principle of Solid Phase Sandwich ELISA technique (Quantikine, USA), expressed in ng/ml. Total RNA was extracted from the skin biopsy using RNA extraction kit provided by Qiagen extraction kit. RNA purity and quantity were measured by Nanodrop. Two sets of primers were used for amplification of PRLR and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as housekeeping gene [Table 1]. The polymerase chain reaction products were quantitated by using a quantitation kit (from Promega Corporation, Madison, WI, USA).

**Statistical analysis**

All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows. Data were statistically described in terms of range, mean ± standard deviation, frequencies (number of cases), and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student’s t-test for independent samples in comparing 2 groups and one-way analysis of variance test when comparing more than 2 groups. For comparing categorical data, Chi-square test was performed. Exact test was used instead when the expected frequency is <5. Accuracy was represented using the terms sensitivity and specificity. Receiver operator characteristic (ROC) analysis was used to determine the optimum cutoff value. Correlation between various variables was done using Pearson moment correlation equation for linear relation. *P*<0.05 was considered statistically significant.

**Results**

The study included 30 patients; 16 (53.3%) males and 14 (46.7%) females with AA, whose age ranged between 18 and 46 years with a mean of 29.4 ± 8.82 years. The disease duration ranged between 1 month and 96 months, with a mean of 16.63 ± 20.38 months. All the patients had scalp AA with variable extent of lesions. The SALT score ranged from 3 to 70 with a mean of 17.87 ± 16.41. Seventeen patients (56.7%) had associated precipitating factor in the form of psychic stress.

Twenty individuals (9 [45%] males and 11 [55%] females) whose age ranged between 18 and 45 years with a mean of 29.30 ± 8.23 years served as controls. There was no significant difference between patients and controls in terms of age and sex distribution (*P*=0.96 and 0.56, respectively).

**Serum prolactin level and tissue expression of prolactin receptor**

On comparing the serum prolactin level between the patient and the control groups, no significant statistical difference was found (*P*=0.56). Among the patients, the level of serum prolactin ranged between 3.3 and 22.1 ng/ml with a mean of 9.44 ± 4.90 ng/ml whereas within the control group, the level of serum prolactin ranged between 2.2 and 18.2 ng/ml with a mean of 8.67 ± 4.07 ng/ml [Figure 1].

| Gene            | Primers (5’→3’)                           | Product (bp) | Genbank     |
|-----------------|-------------------------------------------|--------------|-------------|
| GAPDH           | Forward: GCAAGTCCAGGCACAG                 | 249          | AJ431207    |
|                 | Reverse: GGTTACGCCCATACAA                |              |             |
| PRLR            | Forward: TATCCAGGACAGAAATACC             | 147          | AF041257    |
|                 | Reverse: AGAAAGGACGCCACAAA               |              |             |
| GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, PRLR: Prolactin and prolactin receptor | | | |

Table 1: Real-time polymerase chain reaction primer sequences and polymerase chain reaction product size for glyceraldehyde-3-phosphate dehydrogenase, prolactin and prolactin receptor
On the other hand, the mean tissue level of prolactin receptor was significantly higher in the lesional skin of patients (18.1–82.1 pg/g with a mean of 49.90±18.29 pg/g) compared to the normal skin of controls (10.7–69.9 pg/ml with a mean of 37.07±19.30 pg/g) (P=0.02) [Figure 1].

To determine the cutoff limit for prolactin receptor level that would differentiate between normal and AA skin, we performed ROC analysis. A level of 25.6 pg/ml was determined as the cutoff point, with a sensitivity of 83.3% and specificity of 50% (95% CI: 0.54–0.85, P=0.02) [Figure 2].

A statistically significant positive correlation was found between the tissue level of prolactin receptor in the patients and their SALT score (r=0.36, P=0.045). No statistically significant correlation was found between the tissue level of prolactin receptor and the disease duration (r=0.31, P=0.09).

**Discussion**

The answer provided by the current study to the proposed question around the possible role played by prolactin in AA is that this role is most probably played through the prolactin receptor rather than serum prolactin level itself. This was evident through the detection of a significantly increased expression of prolactin receptor in the patient group in comparison to control, with no significant difference in the prolactin serum level between the two groups.

To the best of our knowledge, this is the first report on the importance of prolactin receptor in AA. The findings of the present study allowed us to hypothesize that the role of prolactin in AA could simulate that of androgen in female pattern hair loss where several studies documented normal serum androgen level with increased number and sensitivity of androgen receptors in such patients.[14,15]

The absence of a significant difference in the serum level of prolactin in patients in comparison to controls detected in the current study comes in accordance with that documented by two previous studies.[9,10] Accordingly, both studies denied a possible role of prolactin in the pathogenesis of AA, something we could not state owing to the further studying of the prolactin receptor. On the contrary, two other studies documented a significantly elevated level of serum prolactin in AA patients compared to controls,[11,12] and that serum prolactin level was significantly correlated with disease severity.[11]

In the current study, we postulate that the role of prolactin in AA could be explained by the increased number of prolactin receptor and thereby increased influence of prolactin on such patients.

Prolactin and AA could be connected with each other through various immunological pathways. Prolactin for one promotes the survival, proliferation, and differentiation of CD4− and CD8− thymocytes into CD4+ and CD8+ cells.[14] These CD4+ and CD8+ cells are clustered at a high density around the anagen hair bulb in AA.[17] The immune privilege collapse that takes place in AA induces expression of MHC class I molecules on follicular cells, leading to the induction of both CD8 positive cytotoxic cells and MHC class II molecules, leading to induction of CD4 helper, and then the downstream autoimmune phenomenon with generation of autoreactive T-cells.[18]

Moreover, prolactin activates the proliferation and lytic function of the natural killer cells.[19] These natural cells are highly active in patients with AA where hair follicles have a decreased capacity to suppress their undesired cell activity.[20] Many perifollicular CD56+ natural killer (NK) cells with high expression of the NK cell activating receptor NKG2D are present in AA lesions but not in normal scalp.[17] At the same time, the ligand for NKG2D, MHC class I chain-related protein, was found to be expressed at very high levels in the proximal outer root sheath, the dermal papilla, and the connective tissue.
sheath of AA hair follicles.[21] The immune privilege hair follicle collapse that occurs in AA leads to NK2D+ NK cell attack which can recognize autoantigens that result in apoptotic responses and hair loss.[22]

Add to this, the ability of prolactin to stimulate the synthesis and release of immunomodulating cytokines and lymphocyte-activating factors, especially IL-1 which was found to be highly expressed in patients with AA may have a role in causing damage to the hair follicle.[23] Studies have shown that IL-1 is a very potent inducer of hair loss and a significant human hair growth inhibitor in vitro.[24] In human scalp, areas affected by AA have an excessive expression of IL-1β particularly at the early stages of the disease, while susceptibility to the disease and severity are determined by polymorphisms of the IL-1α.[25] In addition to this, prolactin increases the synthesis of IFN-γ,[26] which is the main cytokine known to be aberrantly expressed in AA through a CD4+ Th1-mediated response. Among several actions, it deprives dermal papilla cells from their ability to maintain anagen hair growth.[27] Moreover, prolactin also increases the synthesis of TNF-α[28] which plays a role in the pathogenesis of AA.[29]

From all the abovementioned points, we can assume the relation between prolactin and AA being explained by the positive result that the present study has reached. The detection of a significant positive correlation between prolactin receptor and the SALT score indicates that the prolactin receptor is actually related to the disease severity. However, no similar correlation could be detected between the receptor and the disease duration denying their role in the disease chronicity.

Finally, we can conclude that prolactin plays a role in AA and that this role is most probably through the prolactin receptor rather than serum prolactin itself. Moreover, the prolactin receptor expression is positively related to the disease severity.

This study reopens the doors for a deeper understanding of AA with highlighting a positive role played by prolactin in its pathogenesis, evident by the significant increase in prolactin receptor expression in AA. In the last few years, prolactin receptor antagonists have been widely incorporated in the treatments of different diseases including hyperprolactinemia, breast cancer, and prostatic cancer.[10] The use of prolactin receptor antagonists may be of beneficial therapeutic effect in AA as well, especially in severe cases, a suggestion that needs further elaborative work.

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Conflicts of interest
There are no conflicts of interest.

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