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# Serum Level of Programmed Death Ligand1 in Alopecia Areata Patients in Relation to Disease Severity

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**Abstract**

There is an estimated lifetime risk of 1.7% for sufferers of autoimmune hair loss condition Alopecia areata (AA). Alopecia areata (AA) is a common symptom of AA, and it may affect any part of the body. Prevalence among young and middle-aged individuals ranges from 0.9% to 6.9 percent. Most individuals (50–60 percent) acquire AA before they are 20 years old, however the condition may arise at any time. However, genetic and environmental variables have been shown to have a significant role in the development of AA. It has been suggested that the collapse of the immune privilege of the hair follicle is a crucial factor in the development of Alzheimer's disease. As a 40kDa type 1 transmembrane protein, programmed death-ligand 1 (PD-L1) plays a crucial role in dampening the adaptive arm of the immune system, according to some researchers. Additionally, PD-L1 helps to build immunological privilege sites. PD-L1 levels in patients with AA were studied to see whether they were associated with disease severity. Researchers used 60 men and women with AA and 20 healthy volunteers for this case-control research at the Dermatology and Andrology Clinic at the Benha University Hospitals. An ELISA method and the SALT score were used to determine the severity of illness in the patients' group for programmed death ligand 1 serum levels. There were no significant variations in age or sex between the groups in this investigation. There are no major variations in family history between the two groups. In 10% of instances, a member of the patient's family had a history of alcoholism. SALT scores were used to determine severity. There was a significant difference between the AA group and the control group in terms of PD-L1 levels in the present research. SALT score was positively correlated with PD-L1 level. PD-L1 was shown to be an independent predictor of AA severity. The findings of this research show that PD-L1 has a role in the aetiology of AA. As an indicator of the severity of the condition, PD-L1 might be employed. In addition, this research might lead to the development of novel treatment options for AA patients.

**Key words:** Serum Level of Programmed Death Ligand1, Alopecia Areata.

## 1. Introduction

Hair loss disorder alopecia areata (AA) has a reported lifetime risk of 1.7 percent. If you have alopecia areata, you may see patches of hair loss on any part of your scalp or body [1]. Prevalence among young and middle-aged individuals ranges from 0.9% to 6.9 percent. The onset of AA may occur at any age, although 50–60% of patients are under the age of 20 when they are diagnosed [2].

Small, coin-sized patches to entire scalp hair loss (alopecia totalis) or complete scalp and body hair loss (alopecia universalis) (alopecia universalis). There is no visible scarring or inflammation on the skin. Anxiety, despair, or a decrease in quality of life are all possible side effects of hair loss in alcoholics [3].

However, genetic and environmental variables have been shown to have a significant role in the development of AA [4].

Relative immune privilege is an important property of the hair, which is mostly achieved by suppressing surface molecules essential for presenting autoantigens (i.e., MHC class I) to CD8+ T cells, and by creating an inhibitory local signalling environment [5]. The loss of the hair follicle's immunological privilege has long been recognised to be a crucial factor in the development of AA [6].

As a 40kDa type 1 transmembrane protein, programmed death-ligand 1 (PD-L1) has been speculated to play a major role in suppressing the adaptive arm of the immune system in certain situations, including during the course of pregnancy, tissue allografting and the development of autoimmune and other disease states. In normal circumstances, the adaptive immune system responds to antigens that are linked to the activation of the immune system by external or internal threats. Antigen-specific CD8+ T cells and/or CD4+ helper cells are clonally expanded as a result. An inhibitory signal is sent through the Immunoreceptor Tyrosine-Based Switch Motif (ITSM) when PD-L1 binds to the inhibitory checkpoint protein PD-1 [7].

Additionally, PD-L1 helps to build immunological privilege sites. Skin samples from healthy people have been shown to contain PD-L1+mast cells. In contrast, the amount of lesional AA skin cells seemed to be decreased compared to healthy skin [8].

As a primary objective, the research sought to determine the correlation between the serum PD-L1 concentration in AA patients and their illness severity.

## 2. Patients and Methods

### Type of the Study

This study was conducted as case- control study.

### Study Population

A total of 60 individuals with AA were studied in this research (Group A). As a control group, 20 healthy persons of the same age and sex were included in the study (Group B).

Benha University Hospital's Dermatology and Andrology Outpatient Clinic chose all participants.

Consideration of Administrative Design and Ethical Issues.

The Benha Faculty of Medicine's local ethics committee authorised the research. Prior to enrolling in the trial, informed permission was acquired from each participant.

Criteria for Inclusion

Clinical and dermoscopic investigations were used to identify patients with AA on the scalp. SALT (Severity of Alopecia Tool) scores were used to determine the lesions' severity [9].

**Exclusion Criteria**

**Patients with AA suffering from:**

- Localized scalp infections/inflammation.
- Inflammatory skin disease.
- Autoimmune diseases (RA) or psoriasis.
- Systemic disease.
- Malignant tumors.
- Pregnant and lactating female patients.
- Patients on topical and systemic therapy for 4 weeks prior to study.

**Methods:**

**All patients were subjected to the followings**

**Full history taking including:**

**Personal history:**

- Patient's name, age, sex and occupation (indoor or outdoor).

**Present history:**

- Onset, course and duration of the AA.
- Number of patches.
- The previous medications the patient had before for the disease.

**Past history:**

- Past history of prior episodes of AA.
- History of systemic diseases as diabetes mellitus, systemic lupus erythematosus or thyroiditis.

**Family history:**

- Alopecia areata and other skin or systemic diseases.

**Complete general examination:**

- Body mass index (BMI), waist circumference, systolic and diastolic blood pressure.

**Clinical examination:**

**Complete general examination**

- Complete chest and abdomen examination were done to exclude other autoimmune or systemic diseases.

**Clinical assessment of the skin (Local examination)**

- Sites, number, morphology and configuration of AA lesions were recorded.

**Gradingof alopecia areata:**

- Severity of AA lesions was assessed using SALT score. SALT score (Fig. 1) is a mathematical approach to determine percentage of hair loss in the scalp.

**The scalp is divided into the following 4 areas:**

- Vertex: 40% (0.4) of scalp surface area.
- Right profile of scalp: 18% (0.18) of scalp surface area.
- Left profile of scalp: 18% (0.18) of scalp surface area.
- Posterior aspect of scalp: 24% (0.24) of scalp surface area.

Percentage of hair loss in any of these areas is the percentage hair loss multiplied by percent surface area of the scalp in that area.

SALT score is the sum of percentage of hair loss in all the above-mentioned areas as shown in Oslen/Canfield tool [9].

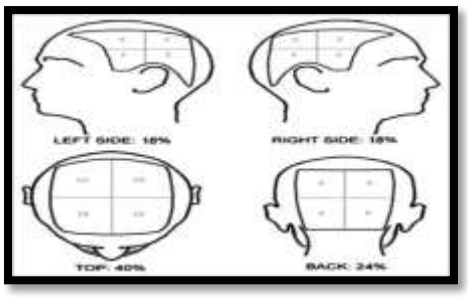


**Fig. (1)** SALT score calculation [9]

**Hair pull tests**

Anagen effluvium, anagen effluvium, telogen effluvium, and loose anagen syndrome may all be monitored using the hair pull test. An acute case of hair loss and a serious medical condition are the ideal conditions for conducting a hair pull test. In patients with long-term conditions, the hair pull test should not be used for essential choices because of the limited sensitivity and substantial interobserver variability of the test (10).

With thumb, index and middle fingers grip 50 to 60 hairs from the peripheral alopecic patch as well as three distinct perilesional locations. Strong yet gentle pulls were used to remove hair from the scalp and along the hair shafts. A positive pull test is one in which you can take out at least six hairs, indicating that you are experiencing hair loss (11).

**Experiments in the lab:**

Kits for Human Programmed Cell Death Protein 1 Ligand 1 (ELISA) were used to estimate serum PD-L1 concentrations.

**Analytical Methods**

In order to analyse the data, a statistical tool for social science (IBM Corp. Released 2017) was used to edit, code, and tabulate it. Version 25.0 of IBM SPSS Statistics for Windows. IBM Corp., Armonk, New York.). The data was displayed and analysed in accordance with the kind of data that was collected for each metric.

## 3. Results

**Demographic,anthropometric data and family history**

The mean age of AA group was 25.9 years, they were 37 males (61.7%) and 23 females (38.3%). In addition to 20 healthy control group of matched age and gender. Tab (1)

No significant differences were found between both groups regarding BMI and family history (p>0.05 for each).

**Table (1)** Comparison of demographic, anthropometric data and family history between cases and control groups.

| | | Control N=20 | | AA N=60 | | p |
|---|---|---|---|---|---|---|
| **Age (years)** | **mean±SD** | 24.4 | 5.2 | 25.9 | 7.6 | 0.566 |
| **Males** | **N, %** | 14 | 70% | 37 | 61.7% | 0.502 |
| **Females** | **N, %** | 6 | 30% | 23 | 38.3% | |
| **BMI (kg/m$^2$)** | **mean±SD** | 23.9 | 4.7 | 26.2 | 5.5 | 0.100 |
| **Normal weight** | **N, %** | 10 | 50% | 22 | 36.7% | 0.292 |
| **Over weight** | **N, %** | 10 | 50% | 38 | 63.3% | |
| **Positive family history** | **N, %** | 1 | 5% | 6 | 10% | 0.673 |

SD, standard deviation; student t test was used for numerical parameters; Chi square test was used for categorical parameters.

**Correlation of PD-L1 level with other parameters**

PD-L1 level showed significant positive correlation with SALT score (p<0.001); but not with age, BMI or duration (p>0.05 for each). Tab (2)

**Table 2. Correlations of PD-L1 level with age, BMI, duration and SALT score in AA group.**

| | PD-L1 | |
|---|---|---|
| | **r** | **p** |
| **age** | 0.063 | 0.632 |
| **BMI** | -0.229 | 0.078 |
| **Duration** | -0.110 | 0.405 |
| **SALT** | 0.729 | **<0.001** |

Median disease duration was 1 month, ranged from 1 week to 1 year. AA cases had gradual onset in 15% of cases, acute onset in 85% of cases, stationary course in 13.3% of AA patients, progressive course in 86.7% of AA patients. Tab (3 )

**Table 3. Clinical features in all studied AA cases.**

| | | | AA N=60 | |
|---|---|---|---|---|
| **Disease duration (months)** | | **Median (range)** | 1 | (0.25-12) |
| **Onset** | **Gradual** | **N, %** | 9 | 15.0% |
| | **Acute** | **N, %** | 51 | 85.0% |
| **Course** | **Stationary** | **N, %** | 8 | 13.3% |
| | **Progressive** | **N, %** | 52 | 86.7% |
| **Dermoscopic findings** | **Yellow dots** | **N, %** | 49 | 81.7% |
| | **Black dots** | **N, %** | 45 | 75.0% |
| | **coudability sign** | **N, %** | 37 | 61.7% |
| | **exclamation mark hair** | **N, %** | 55 | 91.7% |
| | **short vellus hair** | **N, %** | 14 | 23.3% |
| | **follicular ostia** | **N, %** | 60 | 100.0% |
| **SALT** | | **Median (range)** | 6 | (2-55) |
| **Subclass** | **S1** | **N, %** | 38 | 63.3% |
| | **S2** | **N, %** | 16 | 26.7% |
| | **S3** | **N, %** | 6 | 10.0% |
| **Severity** | **Mild (S1)** | **N, %** | 38 | 63.3% |
| | **Moderate (S2)** | **N, %** | 16 | 26.7% |
| | **Extensive (S3 to S5)** | **N, %** | 6 | 10.0% |
| **History of previous treatment** | | **N, %** | 13 | 21.7% |

-L1 level increased gradually with increased AA severity (p<0.001).tab ( 4).

**Table (4)** Association of PD-L1 level with severity of AA.

| | | PD-L1 | | | | p |
|---|---|---|---|---|---|---|
| | | N | mean | ± | SD | |
| **Severity** | **mild** | 38 | 392.4 | ± | 85.8 | |
| | **moderate** | 16 | 690.6 | ± | 140.8 | **<0.001** |
| | **Extensive** | 6 | 958.1 | ± | 72.6 | |

Higher frequency of follicular ostia was significantly associated with severity (p=0.011). Other dermoscpic findings were not associated with severity (p>0.05). Table (5).

**Table (5)** Association of dermoscopic findings with severity.

| Dermoscopic findings | Mild N=38 | | moderate N=16 | | Extensive N=6 | | p |
|---|---|---|---|---|---|---|---|
| | N | % | N | % | N | % | |
| **Yellow dots** | 28 | 73.7% | 15 | 93.8% | 6 | 100% | 0.154 |
| **Black dots** | 25 | 65.8% | 14 | 87.5% | 6 | 100% | 0.119 |
| **coudability sign** | 25 | 65.8% | 8 | 50% | 4 | 66.7% | 0.519 |
| **exclamation mark hair** | 35 | 92.1% | 14 | 87.5% | 6 | 100% | 0.784 |
| **short vellus hair** | 38 | 100% | 16 | 100% | 6 | 100% | - |
| **follicular ostia** | 5 | 13.2% | 5 | 31.3% | 4 | 66.7% | **0.011** |

AA group showed significantly higher level of PD-L1 when compared to control group (mean=528.5 pg/ml versus 254.7 pg/ml; p<0.001). Table (6).

**Table (6)** Comparison of PD-L1 level among studied groups.

| | Control N=20 | | AA N=60 | | P |
|---|---|---|---|---|---|
| | mean | ±SD | mean | ±SD | |
| **PD-L1 (pg/ml)** | 254.7 | 84.1 | 528.5 | 175.5 | **<0.001** |

SD, standard deviation; student t test was used for numerical parameters.

**ROC curve for PD-L1 levels**

PD-L1 showed excellent AUC (AUC=0.915). At best cut off value of 385.15, sensitivity was 73.3%, specificity was 100%, PPV was 73.3%, NPV was 100%, and accuracy was 80%. Table (7)

**Table (7)** Validity of PD-L1 level for discrimination between AA cases and control groups.

| | PD-L1 |
|---|---|
| **AUC** | 0.915 |
| **Cut off** | 385.15 |
| **Sensitivity (%)** | 73.3 |
| **Specificity (%)** | 100 |
| **PPV (%)** | 73.3 |
| **NPV (%)** | 100 |
| **Accuracy (%)** | 80 |

AUC, area under ROC, OC, receiver operating curve; PPV, positive predictive value; NPV, negative predictive value

**Validity of PD-L1 levels for prediction of AA severity.**

ROC curve of PD-L1 level was conducted for prediction of AA severity (discrimination between mild versus moderate+extensive AA cases) (prognosis of AA).

PD-L1 showed excellent AUC (AUC=0.990). At best cut off value of 499.05, performance characteristics are shown in table (8).

**Table (8)** Validity of PD-L1 levels for prediction of AA severity.

| | PD-L1 |
|---|---|
| **AUC** | 0.990 |
| **Cut off** | 499.05 |
| **Sensitivity (%)** | 100 |
| **Specificity (%)** | 92.1 |
| **PPV (%)** | 100 |
| **NPV (%)** | 92.1 |
| **Accuracy (%)** | 95 |

AUC, area under ROC, OC, receiver operating curve; PPV, positive predictive value; NPV, negative predictive value.

Linear regression analysis was conducted for prediction of AA severity (higher SALT score) using age, gender, BMI, FH, onset, course, duration, PD-L1 level as confounders. Higher PD-L1 level was considered independent predictor of AA severity. Table (9)

**Table (9)** Regression analysis for prediction of factors affecting severity of AA (higher SALT score).

| | β | p |
|---|---|---|
| **Age** | 0.002 | 0.992 |
| **Gender** | 2.032 | 0.643 |
| **BMI** | -0.580 | 0.134 |
| **Positive family history** | 0.500 | 0.944 |
| **Early onset** | 3.915 | 0.117 |
| **Progressive course** | 3.038 | 0.628 |
| **Duration** | 0.424 | 0.570 |
| **PD-L1** | 0.063 | **<0.001** |

B, linear regression coefficient.

## 4. Discussion

Age and gender did not vary significantly across the groups in this investigation. In the AA group, there were 37 men and 23 females, a mean age of 25.9 years (38.3 percent).

The findings of Bhat et al. [12] and Al-Mutairi and Eldin [13] support the current study, which found that the incidence of AA in men was 2.12 times higher than that in women, and that the majority of the patients were younger than 40. A research by El-Morsy et al. [14] comprised 39 individuals with AA of the scalp, with an average age of 24.51 14.26 years standard deviation. Seventy-eight patients (71.8 percent) were men, whereas just eleven (28.1 percent) were women.

It was determined that there were no significant differences between the two groups when it came to their family history. In 10% of instances, a member of the patient's family had a history of alcoholism. Esfandiarpour et al. [15] reported positive family history of AA in 26.5 percent and Wani and Jan [16] found positive family history in 20 percent of the patients, which was in accordance with our findings. ( However, Manzoor and Masood [17] observed that the family history of AA was low in another investigation (1.5 percent ). Ethnic differences and variances in the number of instances (200) may be to blame for the mismatch.

There was a significant (p0.001) positive association between PD-L1 level and SALT score in the present research.

In this research, the majority of AA patients (n = 38; 63.3 percent) had S1 severity grade, followed by 16 patients (26.7 percent) with S2 grade and six patients (10 percent) with S3-S5 severity grade. SALT Score severity (n = 52; 42.62 percent), followed by S2 grade (n = 35; 28.69 percent), S3 grade (n = 17; 13.93 percent), and S4 grade (n = 7; 5.74 percent) were shown to decrease in a declining trend in the research by Rehman et al, [18].

Dermoscopy has been shown to be an effective noninvasive diagnostic technique for AA in the current research.

Yellow spots, black dots, short vellus hair, and exclamation mark hair are all common AA dermoscopic findings [19].

Round or polycyclic dots in yellow to yellow-pink hues may be observed in a variety of sizes and shapes. An infundibular distension filled with keratinous particles and sebum is what these lesions depict [20]. 81% of patients had yellow dots in the present research, which was in accordance with [21, 22] who found that 84% and 81.8 percent of cases, respectively, had yellow dots. In another research by Bains and Kaur [19], however, 61.5 percent of the patients had yellow spots. A research by Bains and Kaur [19] found a low occurrence of yellow spots among Asian patients, which may be due to their yellowish skin tone. There was an increased proportion of yellow dots in the current research due to a smaller sample size and the fact that most of the cases in this study were moderate.

Alopecia areata activity and severity are indicated by the presence of BDs, which are hairs that have been destroyed at the entrance of the hair follicle [23]. For example, Bains and Kaur [19], who discovered that 82.7 percent of patients had black dots, as well as Mane et al. [22] who found that 67.7 percent of patients had black dots, all found comparable findings.

Coudability hairs bend easily, resulting in a noticeable kink. Not only is the presence of these hairs helpful in diagnosing the condition, but they are also indicative of disease activity [24]. Bains and Kaur [19] discovered caudability hair in 36.5 percent of patients in the present research, but in the current study 61.7 percent of cases exhibited coudability sign.

These hairs, also known as exclamation mark hairs or tapering hairs, have a larger diameter at the distal end and a smaller diameter at the proximal end [25]. In the present research, 91.7 percent of AA patients had exclamation mark hair, while Bains and Kaur [19] discovered exclamation mark hair in only 26.9 percent of the patients.. Many patients in this research had just been diagnosed and were now exhibiting symptoms, which led to a greater prevalence rate for exclamation mark hair.

The thin, nonpigmented hairs called "short vellus hairs" were found in 23.3% of the cases in the present investigation, and they were shown to be significantly related with a longer survival time (p=0.003).

Short vellus hair was detected in 57.7 percent of the patients studied by Bains and Kaur [19].

Dermoscopic follicular ostia was shown to be a significant predictor of severity (p=0.011) in this research.

The PD-L1 levels in the AA group were substantially higher than in the control group in the present research (mean= 528.5 pg/ml vs 254.7 pg/ml;

p0.001). AA participants also had significantly higher levels of PD-L2.

In AA-affected mouse skin, Li et al. [26] reported an abundance of PD-L1 expressing cells, which were later revealed to be collagen-producing cells. This was comparable (fibroblasts). Lack of inverse regulation between CD4+ and CD8+ cells that have been infiltrated with PD-L-expressing fibroblasts does not support the co-inhibitory involvement of these ligands in the skin of AA-affected C3H mice To understand why dermal T cell activation and inflammation seem to be correlated with PD-L1 expression, it is necessary to look at the association between PD-L1 and the amount of infiltrating T cells.

sPD-1 and sPD-L1 concentrations in blood samples from SLE patients were shown to be elevated compared to normal healthy controls (P.0001) by Du et al. [27].

This study found that the PD-L1 sensitivity was 73.3 percent and the specificity was 100 percent for predicting AA severity (the difference between mild and moderate+extensive AA patients) (prognosis of AA). sensitivity was 100% and specificity was 92.1 %.

The severity of AA might be predicted using a linear regression model (higher SALT score). PD-L1 was shown to be an independent predictor of AA severity.

PD-L1 has been shown to have a function in the development of AA in this investigation. HF-IP has been linked to its activity, which might be used as a biomarker to predict the onset and maintenance of IP status in tissues and cells [28]. The PD-1 receptor on T-cells transmits an inhibitory signal that inhibits cytokine synthesis and proliferation while promoting the death of T-cells [29].

The pathophysiology of AA is influenced by these effects. There is evidence that the blood levels of PD-L1 are raised in individuals with autoimmune disease, which suggests that it may have a role in the development and progression of alopecia. Our understanding of its function in the pathophysiology of AA may be strengthened by the fact that it has been implicated in a variety of inflammatory and autoimmune illnesses.

## 5. Conclusion

The findings of this research show that PD-L1 has a role in the aetiology of AA. As an indicator of the severity of the condition, PD-L1 might be employed. In addition, this research might lead to the development of novel treatment options for AA patients.

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