Role of Selectins in Alopecia Areata

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Abstract: Objective: To demonstrate serum concentrations of selectins (L-selectin and P-selectin) in patients with alopecia areata circumscripta (AAC) in comparison to control group. **Background**: The main histopathological features of AAC is a lymphocytic infiltration surrounding hair follicles. L and P-selectin are proved to be indicators of inflammation. There are no studies regarding their role in AAC. **Patients and methods:** Fifty patients with AAC were involved in the study. The patients treated with anti-histaminic or vascular drugs such as heparin or aspirin were excluded from the study as well as patients receiving systemic treatment before 6 weeks from the start of the study. The control group consisted of 20 healthy subjects. The serum concentrations of soluble L-selectin and P-selectin were detected using ELISA method. **Results**: It was found that P selectin level among patients is higher than controls there is statistically significant difference between both groups regarding serum level of P selectin, while L selectin level among patients is lower than controls, but not statistically significant difference. **Conclusions:** This study shows that P-selectin may play an important role in the pathogenesis of AAC and may be a target of future therapies in this disease and L-selectin may not play an important role in the pathogenesis of AAC.
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1. Introduction

Alopecia areata (AA) is a reversible, immunologically mediated disease, causing death of hair follicles and eventually hair loss. Etiology of the disease has not been clearly understood (**1**). Histopathologic examination revealed that damage to the hair follicle occurs along with lymphocytic infiltration. Another pathogenetic factor is related to decreased vascular supply to hair follicles with a crucial role of selectins (**2**). L-selectin and P-selectin are considered the most important indicators of inflammation (**3**). Significance of selectins was discovered in various dermatological diseases such as psoriasis, systemic lupus erythematosus, urticaria, eczema and lichen planus. There are still few studies demonstrating the role of P & L-selectin in patients with AAC (**4**).

2. Material and methods

This case-control study was conducted on 70 subjects, 50 alopecia areata circumscripta (AAC) patients and 20 healthy subjects as a control group. Cases were selected from the Dermatology Outpatient Clinic, Menoufia University Hospital, while control samples were collected from the hospital staff during the period from February2016to January2017. A written consent approval was obtained from research ethical committee of faculty of medicine Menoufia University from every subject before the study initiation.

Exclusion criteria:

Any case subject with one or more of the following was excluded from the study:

* Patients with a history of systemic treatment (corticosteroid, phototherapy, cyclosporine A) before 6 weeks of the study.

* Patients taken antihistamines or vascular drugs (e.g. vitamin K antagonists, heparin, aspirin) before 6 weeks of the study.

All cases in this study were subjected to:

1- Full history taking:

Personal history: name, age, sex.

- History of the present illness: duration of the disease and number of episodes of disease.
- Family history of similar conditions.

1. General and dermatological clinical examination:

- General examination was done to exclude associated systemic diseases.
- Dermatological examination for determination of the site of disease and nail affection.

Assessment of serum p-selectin levels and L-selectin.

Principle of the Assay:

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti- P-Selectin

polyclonal antibody was pre-coated onto 96-well plates. And the biotin conjugated anti- P-Selectin polyclonal antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and wash with wash buffer.

Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the P-Selectin amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of P-Selectin can be calculated.

Kit components

1. One 96-well plate pre-coated with anti-Human P-Selectin antibody.
2. Lyophilized P-Selectin standards: 2 tubes (10 ng / tube).
3. Sample / Standard diluent buffer: 30ml.
4. Biotin conjugated anti-Human P-Selectin antibody (Concentrated): 130pl. Dilution: 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC) (Concentrated): 130pl. Dilution: 1:100.
7. ABC diluent buffer: 12ml.
8. TMB substrate: 10ml.
9. Stop solution: 10ml.
10. Wash buffer (25X): 30ml.

Statistical analysis

Results were collected, tabulated and statistically analyzed by an IBM compatible personal computer with SPSS statistical package version 20 (SPSS Inc. Released 2011. IBM SPSS statistics for windows, version 20.0, Armnok, NY: IBM Corp.).

Two types of statistical analysis were done:

a) Descriptive statistics e.g. was expressed in: Number (No) percentage (%) mean (x) and standard deviation (SD).

b) Analytic statistics e.g.

- T-test; is a test of significance used for comparison of quantitative variables between two groups of normally distributed data, while Mann Whitney's test was used for comparison of quantitative variables between two groups of not normally distributed data.

Pearson correlation was used to show correlation between two continuous normally distributed variables while Spearman correlation was used for not normally distributed ones.

-Chi-square test ($\chi 2$); was used to study association between qualitative variables. Whenever any of the expected cells were less than five, Fischer"s Exact test was used.

P- value of < 0.05 was considered statistically significant.

3. Results

It was found that P selectin level among patients is higher (Mean ± SD 987.32 ± 249.689) than controls (Mean ± SD 720.69 ± 237.9) there is statistically significant difference between both groups regarding serum level of P selectin (P=<0.001), while L selectin level among patients is lower (Mean ± SD 139.65 ± 53.61) than controls (Mean ± SD 145.46 ± 55), but not statistically significant difference (P=0.765) (**Table1**).

Table 1: Comparison of serum level of P & L selectin among patients and controls

Serum level of:	Studied total group N= 70		Test of significance	P value
	Patients n=50	Controls n=20		
P selectin Mean ± SD	987.32 ± 249.689	720.69 ± 237.9	**t** =4.09	<0.001**S**
L selectin Mean ± SD	139.65 ± 53.61	145.46 ± 55.15	**U**= 0.299	0.765 NS

t means student t test S means significant

It was found that there is statistically significant negative correlation between age and serum Level of p selectin among the patients, (r= -0.403, P=0.004) (**Table2**).

Regarding the sex distribution in our patient group, (31 male and 19 female), male alopecia areata patients recorded significant higher (p=0.034) serum p-selectin level than female patient (967.48± 284.86 vs 826.64±234.59). However, L-selectin serum level was insignificantly lower in male (138.65±49.06) than in female (145.29±60.76) (P=0.666) (**Table3**).

The evaluated selectin serum levels in adults vs children Patients with alopecia areata revealed that, children with alopecia areata had elevated serum levels of both types of selectin p & L mean ± sd 1112.17± 211.02 and 153.68±62.42 respectively) than adult patients (mean ± sd 904.09±241.35 and 130.30±45.58 respectively). This increase was significant regarding P (p=0.003) but not L-selectin serum levels (p=0.178) (**Table4**).

Table 2: Correlation between age and serum level of p selectin among the patients:

	Age	
	(r)	P value
Level of p selectin	-0.403	0.004 S

- S Means significant

Table 3: comparison between male and female patients regarding serum level of P & L selectin

	Sex N=50		test	P value
	Male n= 31	Female n= 19		
level of p selectin mean± SD	967.48± 284.86	826.64±234.59	**t=** 2.170	0.034 S
level of L selectin mean± SD	138.65±49.06	145.29±60.76	**U=** 0.432	0.666 NS

Table 4: comparison of serum level of P and L selectin between both age groups among patients:

	Age group N= 50		Test	P value
	Adult n=30	Child n=20		
level of p selectin Mean± SD	904.09±241.35	1112.17± 211.02	**t=** 3.14	0.003 S
level of L selectin mean± SD	130.30±45.58	153.68±62.42	**U=** 1.35	0.178

4. Discussion

Alopecia areata (AA) is a common form of non-scarring alopecia of the scalp and/or body, distinguished by hair loss without any clinical inflammatory signs.

The prevalence was calculated at 0.1-0.2% with a lifetime risk of 1.7%. It can occur at any age. Patchy AA is the most common type (**5**).

The histological examination of AA patients shows large cell infiltration surrounding the hair follicles with a high number of T cells and other inflammatory cells.

It has been found within bald skin the presence of inflammatory infiltrate also around blood vessels with a thickening of their walls (**6**).

The pathogenesis of AA is not clearly understood. An important pathogenic finding is related to elevated serum concentrations of selectins, which are known as adhesion molecules responsible for lymphocyte migration through the vessel wall and may support vascular theory of alopecia (**7**).

The selectin family formed of three closely related cell surface molecules located in leukocytes (L-selectin), platelets (p-selectin) and vascular endothelium (E-and P-selectin).

The selectins all have a similar structure consisting of an amino terminal calcium-dependent lectin domain, an epidermal growth factor-like domain, and a number of short consensus repeat domains (**8**).

In our research, we focus on P & L-selectin. L-selectin is a key adhesion molecule that regulates both the migration of leukocytes at sites of inflammation and the recirculation of lymphocytes between blood and lymphoid tissues.

L-selectin has an enzymatic cleavage site that results in releasing of L-selectin from the cell surface after leukocyte activation (**9**)**.** Cleavage is important for accurate L- selectin expression and appropriate leukocyte migration.

P-selectin, one of the selectin family of adhesion molecules and its ligand PSGL-1 are located on platelets and leukocytes and play a vital role in their adhesion and activation during ongoing inflammation (**10**).

After stimulation of endothelial cells, P-selectin is expressed on the cell surface and released partially as a soluble protein. This soluble form is increased in disease states, suggesting its identification as an

important marker of vascular pathogenesis of the disease.

The biopsy taken from AA patients revealed the presence of blood clots in the light of capillaries as well as thickening of their wall. This may lead to impair vascular supply to hair follicles and so alopecia areata (**11**).

P-selectin has been studied in diseases of chronic stimulation of endothelial cells, like in vascular thrombosis, acute myocardial infarction and stroke**.**

The aim of this work was to evaluate serum concentrations of L-selectin & P-selectin in patients with AA compared to the control group in an attempt to assess the role of P- and L-selectin in the pathogenesis of alopecia areata and to study the associations, if present, between their elevated levels and the clinical data of the disease. The study recruited 70 subjects, 50 alopecia areata circumscripta (AAC) patients and 20 controls. All patients were subjected to detailed history taking and examination. Venous blood samples were taken from all subjects to assess serum L & P selectin levels.

In the present research, we found that L-selectin level among patients is lower than controls. However, this decreased level could not reach level of significance (P=0.765). While P-selectin level among patients is statistically significant elevated than controls (P=<0.001). This significantly higher level of P-selectin in the serum of AA patients may confirm the theory of vascular pathogenesis of AA.

It has been found that Comparison of serum concentrations of L-selectin between AAC patients and healthy subjects showed no statistically significant difference (p=0.0543). While comparison of serum concentrations of P-selectin between AAC patients and healthy subjects showed that serum level in patients was higher than controls and the difference was statistically significant (p=0.015). So, our study was in agreement with the other study regarding serum level of P & L-selectin in AAC patients (**12**). This may provide evidence about the role of P-selectin in alopecia areata pathogenesis.

In current study, there was significant negative correlation between age and serum level of p-selectin among the diseased group. While there was non-significant negative correlation between age and serum level of L-selectin,**.** In current study, the evaluated selectin serum levels in adults vs children patients with alopecia areata revealed that, children with alopecia areata had elevated serum levels of both types of selectin P and L (1055.12± 257.07 and 148.21±57.08 respectively) than adult patients (831.15±250.17 and 137.48±52.02respectively). This increase was significant regarding P**,** but not regarding L selectin serum levels.

Regarding the sex distribution in our patient group, (31 male and 19 female), male alopecia areata patients recorded significant higher serum p-selectin level than female patient (967.48± 284.86 vs 826.64±234.59). However, L-selectin serum level was insignificantly lower in male (138.65±49.06) than in female (145.29±60.76).

Based on the other study and the significant elevated serum p-selectin level in AA patients than controls in the current study, p-selectin may have a role in the aetiopathogenesis in AA. P-selectin functions as a cell adhesion molecule on the surfaces of activated endothelial cells, which line the inner surface of blood vessels.

Activated endothelial cells express P-selectin, which binds PSGL-1 on leukocytes and monocytes (**13**). This interaction is responsible for the recruitment of inflammatory leukocytes to thrombi. An additional function of P-selectin is in the recruitment of monocyte-derived microparticles, which are a rich source of the blood-clotting element 'tissue factor', to the forming thrombus (**14**). This may lead to formation of blood clots in the capillaries of the hair follicles affected as well as thickening of their wall. This may lead to partial occlusion of their lumen and shortage of the blood supply to hair follicles affected leading to alopecia (**15**).

Conclusion

P- Selectin may play an important role in the pathogenesis of AA through imparing angiogenesis of the hair follicle, while L-selectin may not play an important role in the pathogenesis of this disease.

Conflict of interest

The authors declare no conflict of interest.

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