International Journal of Dermatology Research

ISSN Print: 2664-6471
ISSN Online: 2664-648X
Impact Factor: RJIF 5.69
IJDR 2025; 7(1): 88-95
www.dermatologyjournal.in
Received: 10-04-2025
Accepted: 15-05-2025

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Evaluation of neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, mean platelet volume, CRP and ESR with disease severity in patients with alopecia areata

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DOI: https://www.doi.org/10.33545/26646471.2025.v7.i1b.59

Abstract
Background: Alopecia areata (AA) is an autoimmune and inflammatory disease that usually affects the scalp, bearded area, eyebrows, eyelashes, and other hairy areas of the body. It is mostly diagnosed clinically based on clinical history, physical examination, and trichoscopy. In recent years, novel inflammatory markers derived from hematological tests have been introduced.
Objective: To evaluate the relationship of neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, mean platelet volume, ESR, and C-reactive protein with the severity of alopecia areata.
Methodology: A total of 50 patients with AA were included in group A and another 50 healthy individuals were included in group B according to inclusion & exclusion criteria after getting informed written consent. The severity of AA was evaluated by the SALT score.
Result: The mean age of the AA patients was 26.96±6.09 years, wherein the maximum patients were aged between 21-30 years. AA patients had significantly higher mean platelet volume (10.75±1.55 vs 10.16±1.20 fL, p=0.036, <0.05) and CRP (5.12±3.61 vs 3.97±1.82 mg/dL, p=0.048, <0.05) than healthy individuals. The mean SALT score of AA patients was 41.22±27.60. The MPV and CRP had a significant moderate positive correlation with the severity of alopecia areata (p<0.05). NLR, PLR, and ESR had no such significant association with the severity of AA (p>0.05).
Conclusion: This study concluded that MPV and CRP is a simple and cost-effective investigation that can be used as a severity marker in alopecia areata. However, the meticulous clinical significance of MPV and CRP and their role in disease severity and prognosis, justify further study through inclusive future trials and revolutionary exploration.

Keywords: Alopecia areata, trichoscopy, CRP, ESR, SALT score

Introduction

Alopecia areata (AA) is an organ-specific autoimmune disease in which there is a sudden appearance of sharply demarcated, circular or oval, skin-colored patches of non-scarring hair loss that usually affects the scalp, bearded area, eyebrows, eyelashes, and, less often, other hairy areas of the body [1]. The incidence is nearly the same in all ethnic groups, affecting both men and women equally [2]. The prevalence of alopecia areata varies between 0.05% to 1% across different populations [3]. Clinical history, physical examination, and trichoscopy are the main approaches used to diagnose alopecia areata; however, in circumstances where a diagnosis is indecisive, a scalp biopsy may be carried out [4]. Histopathological features of acute alopecia areata include peribulbar lymphoid cell infiltrate ('swarm of bees') affecting the terminal hair follicles [5]. It is assumed that the pathogenesis of alopecia areata is multifactorial and involves intricate relationships between environmental, genetic, and immune factors [6]. It is thought that it occurs due to loss of immune privilege in hair follicles, autoimmune hair follicle destruction, and activation of inflammatory pathways [7]. Alopecia areata is associated with various autoimmune disorders, such as rheumatoid arthritis (RA), type I diabetes mellitus (DM), vitiligo, systemic lupus erythematosus (SLE), autoimmune thyroiditis, pemphigus vulgaris (PV), pernicious anemia, and celiac disease [8].

Recent research has demonstrated the significance of neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and mean platelet volume (MPV) as markers of systemic inflammation [9]. NLR has developed into a simple and useful method to deliver important information for the prognosis and diagnosis of a variety of diseases [10]. As a marker of platelet activation, mean platelet volume (MPV) has been found to be significant in inflammatory responses [11]. MPV is the simplest method to quantify platelet size, and since the large platelets are more metabolically and enzymatically active, it is considered to be an *in vivo* sign of platelet reactivity [12]. Growth factors generated from active platelets, particularly platelet-derived growth factor (PDGF), act on hair follicle stem cells in alopecia areata, stimulating neovascularization and the formation of new hair follicles [13]. The MPV is a feasible indicator demonstrating the role and activity of platelets, which could be quickly and inexpensively determined from a full blood count (CBC). Several studies have demonstrated the correlation between PLR and many diseases, such as inflammatory diseases, cardiovascular diseases, cancer, and long-term type 2 diabetes [14]. NLR, PLR, and MPV have been identified as potential prognostic markers. The most commonly used acute-phase reactants to indicate the degree of inflammation are the erythrocyte sedimentation rate (ESR) and the C-reactive protein (CRP). CRP has both pro-inflammatory and anti-inflammatory action that induces inflammatory cytokines and tissue factors [15]. ESR reflects the degree of inflammation and correlates with the fibrinogen levels in the plasma [16].

Methods

Cross-sectional comparative study August 2022 to March 2024. A total of 100 patients in the study using a purposive sampling technique. The study was carried out in the Department of Dermatology and Venereology, Bangladesh Medical University (BMU), Dhaka, Bangladesh. A purposive type of sampling technique was applied to collect the sample from the study population during the study period. Sample size was calculated by using the following formula:

$$N = \left(\frac{Z\alpha + Z\beta}{C(r)}\right)^2 + 3$$

The number of patients in Group was 50 and in Group B was 50
Total sample size = Group-A (50) + Group-B (50) = 100
So, the total sample size was 100.

Selection Criteria

Inclusion criteria

- Patients who were diagnosed with alopecia areata both clinically and trichoscopically
- Age ≥18 years
- Both gender
- Apparently healthy individuals

Exclusion criteria

- Patients who had received both topical and systemic drugs for alopecia areata within the previous 3 months
- Pregnancy or lactation
- Smokers and Alcoholics
- Diagnosed cases of any active infection, malnutrition, anemia, immunodeficiency, rheumatologic disease, hematological disease, diabetes mellitus, ulcerative colitis, end-stage renal disease, tuberculosis, or cardiac diseases
- Taking drugs that alter laboratory parameters like, Corticosteroids, Antiplatelet drugs, Lipid-lowering agents, NSAIDs, OCP, HRT, Anti-thyroid medications (Carbimazole, Propylthiouracil), Immunosuppressive or immunomodulator therapy, Anticancer medication, Anticonvulsant (Carbamazepine)

Data Collection Tools: A customized checklist was prepared for each patient. Informed written consent form in Bangla and English. SALT score measuring tool. Tools for physical examination- Hand-held Dermoscope. Laboratory investigation reports. A semi-structured questionnaire. Collection of pictures of the study subjects after obtaining consent

Data processing and analysis

After data collection, all entries were checked, verified for consistency, and analyzed using SPSS version 26 on Windows 10. Exploratory data analysis described the study population. Categorical variables were presented as frequencies and percentages, while continuous variables were summarized using mean and standard deviation. Pearson's correlation analysis evaluated the relationship between inflammatory markers (NLR, PLR, ESR, MPV, and CRP) and alopecia areata severity based on the SALT score. A 95% confidence level was used, and a p-value <0.05 was considered statistically significant.

Ethical consideration: Ethical approval for this study was obtained from the IRB of BMU, Dhaka. Written informed consent was collected from all participants after explaining the study's purpose, procedures, benefits, risks, and their rights to refuse or withdraw. Confidentiality was strictly maintained. Participants were assured full understanding of the study and any potential implications for their well-being before voluntarily agreeing to take part.

Results: A total number of 50 patients with alopecia areata and 50 apparently healthy individuals were included in group A and group B respectively with a male-to-female ratio of 1.7:1. Appropriate statistical techniques were applied as required for data analysis.

Table 1: Distribution of the study subjects by age (n = 100)

Age group (years)	Group-A (n=50)		Group- B (n=50)		Total (n=100)		p-value
	No.	%	No.	%	No	%	
≤20	8	16.0	8	16.0	16	16.0	0.654^{ns}
21-30	29	58.0	25	50.0	54	54.0	
31-40	11	22.0	12	24.0	23	23.0	
>40	2	4.0	5	10.0	7	7.0	
Mean ±SD (years)	26.96±6.09		29.28±8.01		28.12±7.18		0.106^{n}
Range	18-44		19-57		18-57		

The minimum age of the respondents was 18 years and the maximum was 57 years. The mean age of the total population was 28.12±7.18 years. The distribution of patients across age groups did not show any statistically significant variations between the case and control groups (p-value>0.05).

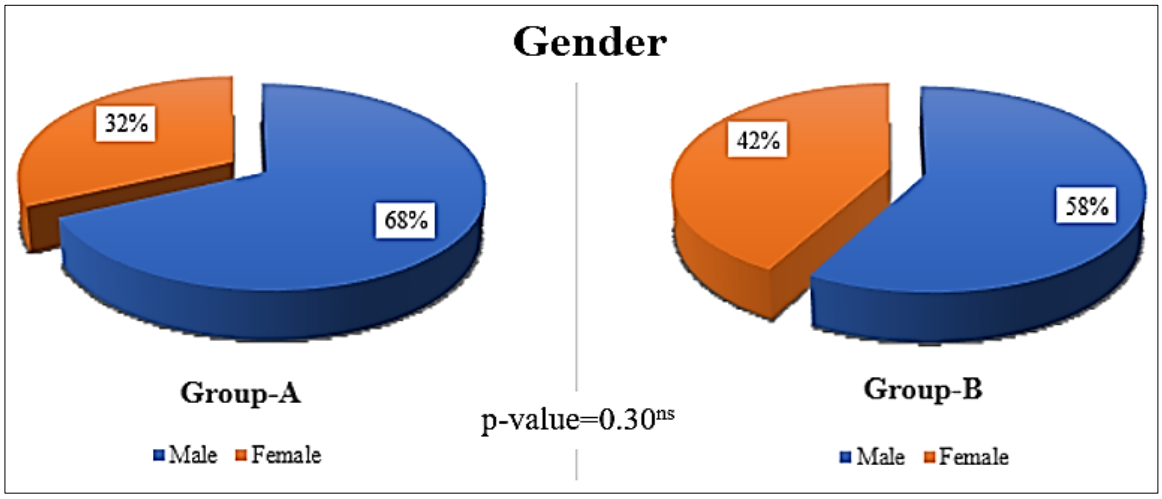

Fig 1: Distribution of the study subjects by gender (n = 100)

The figure-1 statistically similar gender distribution with male predominance (68% in group A and 58% in group B, p=0.30). In this study, the male-to-female ratio was M: F= 1.70:1.

Table 2: Clinical history of the patients with Alopecia areata (n=50)

Variables	Frequency	Percentage
	Mean ±SD (Median, min-max)	
Hair loss	47	94.0
Duration of alopecia areata	4.48±3.64 (3.0, 1-18) months	
Types of alopecia areata		
Patchy AA	49	98.0
Alopecia totalis	1	2.0
Site of involvement		
Vertex	35	70.0
Right profile of scalp	21	42.0
Left profile of scalp	23	46.0
Posterior aspect of scalp	33	66.0

Table 2 reveals that 94% of group A patients experienced hair loss. Group-A patients exhibited patchy alopecia areata in 98% of cases, and alopecia totalis in the remaining 2%. There was no alopecia universalis in the case. The most frequently involved areas were the vertex (70%) and the posterior portion of the head (66%). The disease duration was 4.48±3.64 months on average, with a range of 1-18 months.

Table 3: Dermoscopy findings of the patients with Alopecia areata (n=50)

Variables	Frequency	Percentage
Black dot	45	90.0
Follicular ostia	7	14.0
Exclamation hair	6	12.0
Vellus hair	5	10.0
Yellow dot	3	6.0

Table 3 shows that black dot was the most common dermoscopy finding (90%), followed by decreasing order follicular ostia (14%), exclamation hair (12%), vellus hair (10%), and yellow dot (6%).

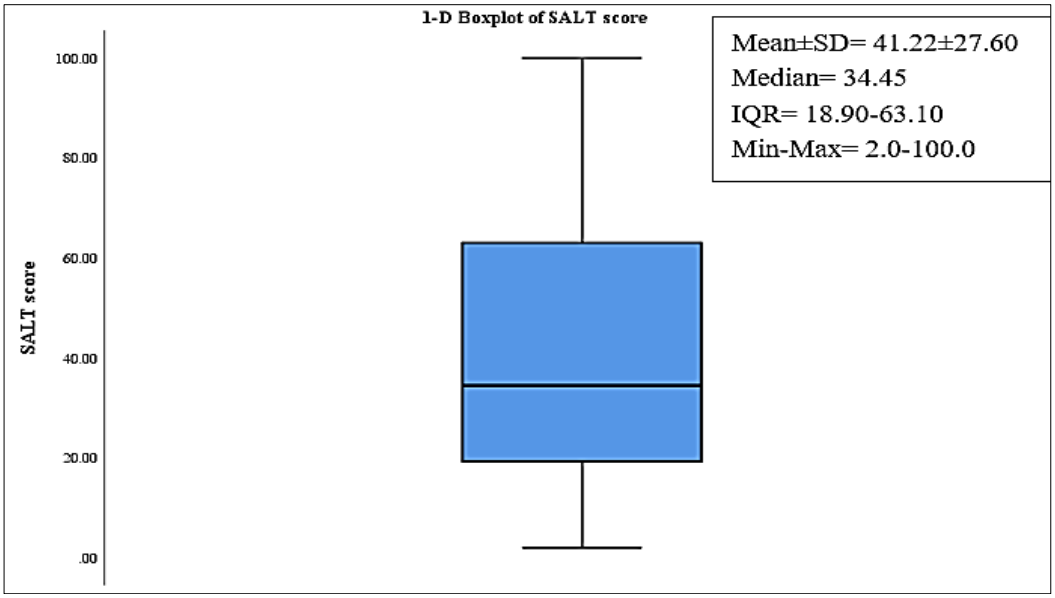

Fig 2: Severity of alopecia according to SALT score among patients with Alopecia areata (n=50)

In this study, the SALT score was used to assess the severity of alopecia areata. Patients with alopecia areata had a mean SALT score of 41.22±27.60, with a range from 2-100.

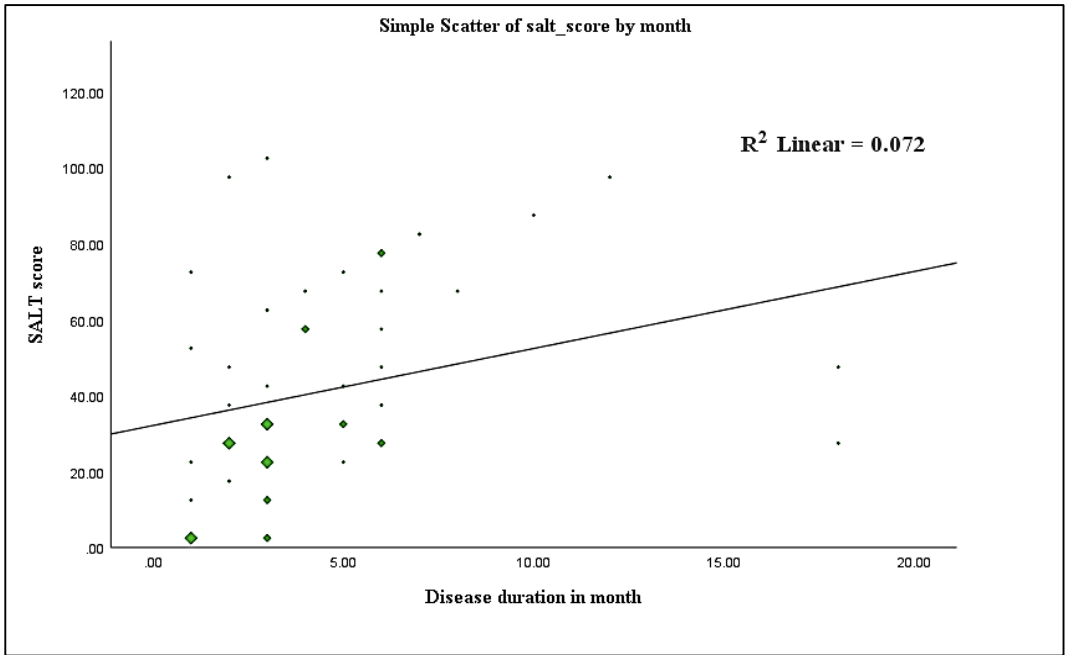

Fig 3: Correlation between disease duration and severity of alopecia areata in patients using scatter plot diagram (n=50)

Pearson correlation model showed that disease duration had a moderate positive relation with the severity of alopecia areata, without statistical significance (r=+0.268, p-value =0.06).

Table 4: Hematological findings of the study populations (n=100)

Variables	Group-A (n=50) Mean ±SD	Group-B (n=50) Mean ±SD	p-value
Total WBC count ($x10^9$/L)	8.30±1.78	8.52±1.92	0.543^{ns}
Absolute neutrophil count ($x10^9$/L)	5.17±1.56	5.16±1.56	0.969^{ns}
Absolute lymphocyte count ($x10^9$/L)	2.57±0.60	2.60±0.63	0.831^{ns}
Platelet count ($x10^3$/µL)	280.74±59.36	289.10±86.29	0.574^{ns}

Table 4 shows that total WBC count, platelet count, absolute neutrophil, and lymphocyte counts were statistically similar between groups (p>0.05).

Table 5: Comparison of values of inflammatory markers (NLR, PLR, ESR, MPV, and CRP) between groups (n=100)

Variables	Group-A (n=50) Mean ±SD	Group-B (n=50) Mean ±SD	p-value
Blood Neutrophil-Lymphocyte ratio (NLR)	2.10±0.74	2.10±0.82	0.996^{ns}
Blood Platelet-Lymphocyte ratio (PLR)	114.97±34.30	115.84±39.31	0.906^{ns}
Mean Platelet Volume (MPV, fL)	10.75±1.55	10.16±1.20	0.036^{s}
ESR (mm in 1st hour)	12.44±8.90	10.32±7.97	0.213^{ns}
CRP (mg/dL)	5.12±3.61	3.97±1.82	0.048^{s}

Table 5 shows that alopecia areata patients had significantly higher mean platelet volume (10.75±1.55 vs 10.16±1.20 fL, p=0.036) and CRP (5.12±3.61 vs 3.97±1.82 mg/dL, p=0.048) than healthy individuals. However, NLR, PLR, and ESR were found to be statistically similar between groups (p>0.05).

Table 6: Correlation of inflammatory markers (NLR, PLR, ESR, MPV, and CRP) with the severity of alopecia areata according to SALT score (n=50)

Variables		SALT score	NLR	PLR	MPV	ESR	CRP
SALT score	*r*	1.0					
	p						
NLR	*r*	+0.223	1.0				
	p	0.120^{ns}					
PLR	*r*	-0.016	+0.576	1.0			
	p	0.909^{ns}	<0.001^{s}				
MPV	*r*	+0.416	+0.124	-0.271	1.0		
	p	0.003^{s}	0.221	0.006^{s}			
ESR	*r*	+0.078	+0.088	-0.122	+0.266	1.0	
	p	0.590^{ns}	0.383	0.227	0.007		
CRP	*r*	+0.609	+0.258	+0.146	+0.162	+0.09	1.0
	p	<0.001^{s}	0.01^{s}	0.147	0.108	0.372	

Table 6 shows that mean platelet volume and CRP had a significant correlation with the severity of alopecia areata (p<0.05). However, NLR, PLR, and ESR had no such correlation with the severity of alopecia areata (p>0.05). Additionally, NLR had a significant positive correlation with PLR and CRP, while MPV had a significant negative correlation with PLR.

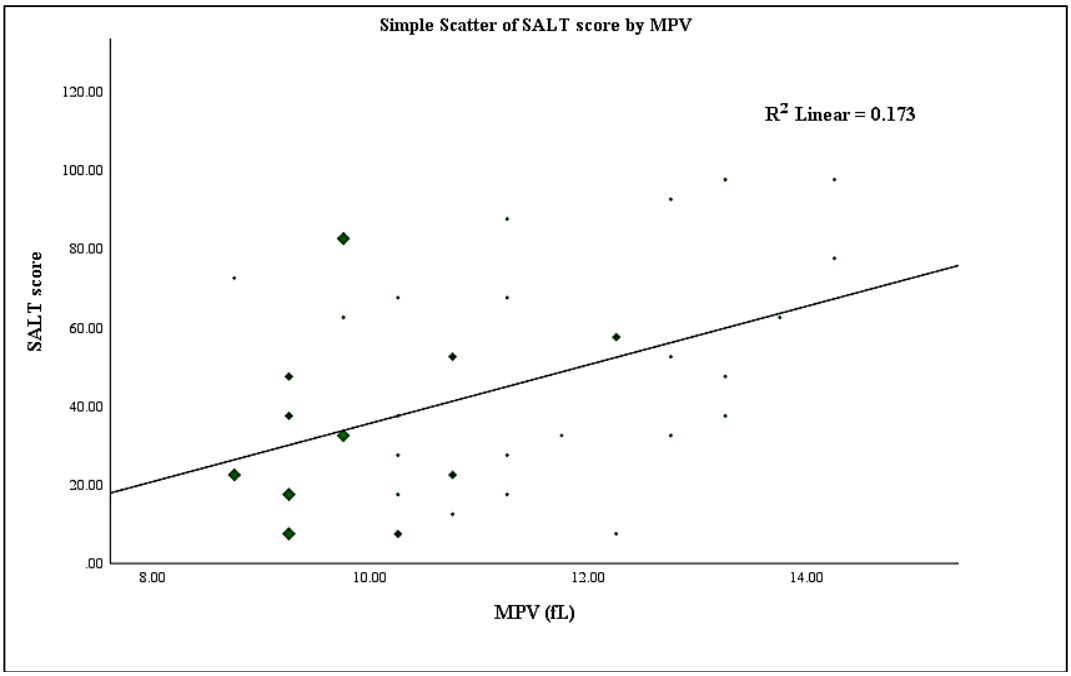

Fig 4: Correlation between MPV and severity of alopecia areata in patients using scatter plot diagram (n=50)

Pearson correlation model showed that MPV had a significant moderate positive relation with severity of alopecia areata according to SALT score (r=+0.416, p value=0.003).

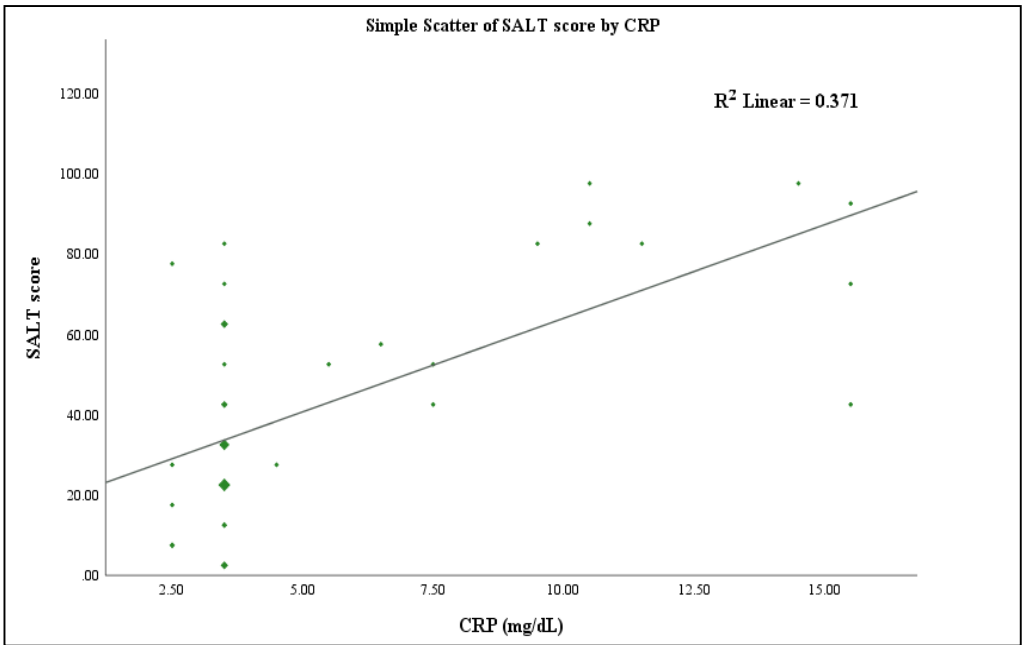

Fig 5: Correlation between CRP and severity of alopecia areata in patients using scatter plot diagram (n=50)

Pearson correlation model showed that CRP had a significant moderate positive relation with the severity of alopecia areata according to the SALT score (r=+0.609, p-value <0.001).

Discussion

This study included 50 alopecia areata patients in total, with an equal number of apparently healthy individuals serving as the comparison group. Given that age and gender characteristics had no confounding influence on the results of the present study, this may offer a sufficient basis for comparison between the two groups. Islamoglu & Demirbras *et al.* [2] included 105 patients with AA and 105 healthy controls in their study. Dere & Gündoğdu *et al.* [7] led a case-control study that enrolled 135 AA patients and 135 healthy controls were enrolled. Sarac *et al.* [17] conducted a retrospective case-control study in which 70 alopecia areata patients and 70 healthy controls participated. Akpolat *et al.* [18] carried out a case-control study involving 141 children, of whom 70 were age and sex-matched healthy children and 71 were patients with alopecia areata. In the present study, the mean age of the patients with alopecia areata was 28.12±7.18 years, wherein the maximum number of patients were aged between 21-30 years (58%), and the mean age of healthy individuals was 28.12±7.18, with the maximum being between 21-30 years. There was no age difference between the two groups. İslamoğlu & Demirbaş *et al.* [2] reported that the mean age for the alopecia areata patient group was 24.97±10.91 years, and for the control group, it was 27.45±10.80 years. In another study conducted by *et al.* [7] the mean age was found to be 34.15±12.43 in the alopecia areata patient group and 34.25±16.86 in the control group. Age distributions were similar for both groups (p=0.958). Likewise, in a study done by Sarac *et al.* [17] the mean age was 31.57±9.92 years in the patient group and 31.51±7.37 years in the control group. There was no significant difference between the groups according to age (P = 0.969). Study conducted by Zakaria *et al.* [19] found that most of the patients with alopec another ia areata were in the 21-30 age group. In the present study, black dots were the most common trichoscopy findings (90%), followed by, in decreasing order, follicular ostia (14%), exclamation hair (12%), vellus hair (10%), and yellow dots (6%). A Study conducted by Mani *et al.* [20] concluded that the most common finding was black dot, seen in 76% of patients. Similarly, a higher incidence of black dots was seen in 51.4% of patients, as stated by Salahuddin, Menon & David *et al.* [21] Black dots are sensitive markers of disease activity as well as severity. These results were consistent with the present study. Conversely, Dias *et al.* [22] found that yellow dots were the most common finding on trichoscopy, seen in 86.6% of patients. The next common finding was black dots, seen in 68.7% of patients. Rossi *et al.* [23] demonstrated yellow dot prevalence in 94.8% of patients. This may be due to various stages of disease activity and is done on non-pigmented scalp skin. In the present study, out of 50 alopecia areata patients, 68% were male and 32% were female, and out of 50 healthy individuals, 58% were male and 42% were female (P value = 0.30). Male and female ratio, M: F = 1.70:1. Males were more common than females in both groups A and B. In this study, the levels of WBC count, absolute neutrophil count, absolute lymphocyte count, and platelet count in both alopecia areata patients and healthy individuals were (8.30±1.78, 8.52±1.92, p-value=0.543; 5.17±1.56, 5.16±1.56, p-value=0.969; 2.57±0.60, 2.60±0.63, p-value=0.831; and 280.74±59.36, 289.10±86.29, p-value=0.574), respectively. Similarly, Dere & Gündoğdu [7] conducted a study and showed that there was no difference in terms of platelet count, lymphocyte count, and neutrophil count between the case and control groups (p-value=0.222, 0.127, 0.915, respectively). In the current study, the means of MPV in patients with alopecia areata were 10.75±1.55, and in the control group were 10.16±1.20; the p-value was < 0.036. Alopecia areata patients had significantly higher means of MPV than healthy controls. Likewise, in the present study, Akpolat *et al.* [18] conducted a study in which 71 children of alopecia areata and 70 age and gender-matched healthy children were retrospectively evaluated. In the alopecia areata group, the mean MPV level was 8.33±1.15, and in the control group, the mean MPV level was 6.33±0.41. The MPV measurements were statistically

significantly higher in the alopecia areata group (p= 0.001; $p<0.01$) [24]. In this study, CRP values were found to be significantly higher in alopecia areata patients than in healthy individuals (5.12±3.61 vs 3.97±1.82, p = <0.048). Like the current study findings, Islamoglu & Demirbas [2] also found that the CRP levels were significantly raised in the alopecia areata patients ($\bar{x}$ = 0.749) relative to those of healthy subjects ($\bar{x}$ = 0.297) (t (211) = 3.496; P =0.001, <0.05) [25] Regarding NLR, PLR, and ESR, this study could not find any significant association between the two groups (2.10±0.74 vs 2.10±0.82, p=0.996, > 0.05; 114.97±34.30 vs 115.84±39.31, p=0.906, >0.05; 12.44±8.90 vs 10.32±7.97, p=0.213, >0.05, respectively). In accordance with the study findings, previous literature observed that there were no statistically significant differences in the mean of NLR and PLR between the two groups (2.00±1.32 vs 1.80±0.68, p=0.114, >0.05; 120.81±59.92 vs 114.22±30.57, p=0.252, >0.05, respectively) [7]. İslamoğlu & Demirbaş [2] investigated the haematological and inflammatory parameters in alopecia areata. They found that NLR and PLR values are statistically similar between alopecia areata patients and healthy controls (t(211) = 0.321; P =0.749 >0.05); t (211) = −1.954; P = 0.52 >0.05, respectively) and ESR was significantly higher in the control group than in the alopecia areata group ($\bar{x}$ = 9.213) (t (211) = −4.559; P =.000 >.05). This result was consistent with the present study. Islamoglu & Demirbas also did not notice any significant correlation between these inflammatory markers and the severity of alopecia areata (P>.05), but they found a significant, positive relationship between the SALT score and disease duration (r = 0.487; P =.000 <.05), which could be attributed to the difference in the number and race of the patients, duration of illness and variation of the assessment tool.

Conclusion

The study concluded that MPV and CRP levels are significantly higher in alopecia areata patients than those of healthy people. Furthermore, a significant, moderate positive correlation was found between mean platelet volume (MPV) and C-reactive protein (CRP) and the severity of alopecia areata. NLR, PLR, and ESR were identical to those of the healthy controls. Therefore, it may be determined that MPV and CRP are simple and easily available biomarkers for evaluating the severity of alopecia areata patients.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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How to Cite This Article
Malabeka S, Jaigirdar MQH, Tahnin NAT, Ahmed MN, Haque MA, Ashadullah SM, Islam MA, Sultana ML. Evaluation of neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, mean platelet volume, CRP and ESR with disease severity in patients with alopecia areata. International Journal of Dermatology Research 2025;7(1):88-95.