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Alterations in Inflammation Markers Due to Disease Activation in Autoimmune Bullous Diseases

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ABSTRACT

Background: In the pathogenesis of autoimmune bullous diseases, there is an underlying autoinflammation against epidermal/subepidermal structures caused by many inflammatory cells.

Aim / Objectives: In this study, we aimed to determine the alterations in inflammatory markers regarding disease activity in autoimmune bullous diseases and to discuss their contribution to the pathogenesis.

Methods: A total of 191 patients with pemphigus vulgaris (PV) and 46 patients with bullous pemphigoid (BP) who were admitted to the outpatient clinic at the Department of Dermatology were included. The mean platelet volume (MPV) values, thrombocyte, eosinophil, and basophil counts, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels prior and following treatment were examined retrospectively from the patients' medical files. A decrease of 75% or more in Autoimmune Bullous Skin Disorder Intensity Score (ABSIS) was considered a remission period.

Results: Among patients with PV, 78 (40.8%) were men and 113 (59.2%) were women. In patients with PV, MPV value, eosinophil, basophil count, and ESR and CRP levels showed a statistically significant decrease during the remission period, whereas alteration in platelet count was not statistically significant. Eighteen (39.1%) of patients with BP were men and 28 (60.9%) were women. In patients with BP, MPV value, eosinophil count, and ESR and CRP levels showed a statistically significant decrease during the remission period. However, platelet and basophil counts revealed no statistically significant alterations.

Limitations: Evaluation of the ABSIS scores of the followed-up patients by different observers due to the long time interval can be considered among the limitations of the study.

Conclusion: Eosinophils, basophils, and thrombocytes to the inflammation in the pathogenesis of PV, whereas eosinophils and thrombocytes may contribute in the pathogenesis of BP. During the activation period of autoimmune bullous diseases, the level of acute-phase reactants is higher than in the remission period.

KEY WORDS: autoimmune bullous diseases, basophil, eosinophil, ESR, MPV

INTRODUCTION

Autoimmune bullous diseases, such as pemphigus vulgaris (PV) and bullous pemphigoid (BP), are characterized by the production of autoantibodies against structural components of the skin and mucous membranes, leading to blistering and erosions. While the exact causes of these diseases are not fully

understood, a combination of genetic and environmental factors are thought to play a role in their development (1).

Acantholysis induced by the binding of autoantibodies to antigens (desmoglein 1 and desmoglein 3) on the epithelial cell surface is thought to underlie the pathogenesis of pemphigus. However, desmogleins are thought to have a minimal role in about one third of patients with pemphigus, and other factors may play a role in the pathogenesis (such as desmocoline 3, desmocoline 4, acetylcholine receptors) (2,3). Clinical features include painful erosions of the oral mucosa, flaccid blisters, and cutaneous erosions (4). In BP, autoantibodies are thought to develop against hemidesmosome components (BP180 and BP230 antigens) that support epithelial-stromal adhesion in the epithelium, leading to subepidermal bulla formation (1). In BP, tense bullous lesions develop on the basis of erythematous or urticarial plaques. Although the lesions are usually located symmetrically on the skin, oral mucosal involvement is less common in patients with BP than in patients with PV (5).

Inflammation is a complex biological response to tissue injury or infection, involving various cellular and molecular components. Alterations in concentrations of many plasma proteins known as acute-phase reactants reflect the presence and intensity of inflammation. The most commonly used measurements for this purpose are the determination of C-reactive protein (CRP) levels and the measurement of erythrocyte sedimentation rates (ESR) (6). Eosinophils, basophils, and platelets are cellular components that also play a role in the inflammatory process. While eosinophils contain molecules in their granules known to play an important role in inflammation and thrombosis, basophils are thought to be the main source of Th2 cytokines (7,8). The role of platelets in inflammation has been studied more extensively than that of eosinophils and basophils. Immunological and non-immunological stimuli can activate and cause the release of a wide range of biologically active mediators and surface expression of functional molecules (9). Mean platelet volume (MPV) is a parameter that is routinely measured during blood count examination and is considered a marker of platelet activation. MPV has been examined as a simple inflammatory marker in several diseases. Although it has been studied in various clinical conditions, confusing results such as an increase in myocardial infarction and cerebrovascular disease and a decrease in rheumatologic diseases have been reported (10).

Overall, the measurement of acute-phase reactants, CRP and ESR, as well as the examination of cellular components such as eosinophils, basophils, and platelets, including MPV, can provide important information about the presence and intensity of inflammation in various diseases.

In our study, we aimed to investigate alterations in MPV values, thrombocyte, eosinophil, and basophil counts, and ESR and CRP levels prior and following treatment in patients with autoimmune bullous disease.

PATIENTS AND METHODS

This study included 191 of 219 patients with PV and 46 of 94 patients with BP admitted to the outpatient clinic at the Department of Dermatology, Ondokuz Mayis University School of Medicine between 2010 and 2020. Patients with refractory autoimmune bullous disease in whom disease could not be controlled with topical therapy and low-dose systemic corticosteroid were included in the study. Patients with a history of atherosclerotic heart disease, drug use affecting platelet function, hematological disease, and malignancy were excluded from the study. For histopathological and direct immunofluorescence examinations, samples were taken with a 4 mm punch from patients who applied to our outpatient clinic. The diagnosis of PV was established with suprabasal detachment, acantholysis, retention of basal keratinocytes along the basement membrane in the dermis (tombstone appearance) in the histopathological examination, and intercellular involvement of IgG (fishnet appearance) on direct immunofluorescence examination. BP was diagnosed with eosinophilic spongiosis, subepidermal bullae on histopathological examination, and linear IgG and/or C3 involvement in direct immunofluorescence examination.

The disease severity of patients with PV and BP was evaluated with the Autoimmune Bullous Skin Disorder Intensity Score (ABSIS) (11). The MPV values, thrombocyte, eosinophil and basophil counts, and ESR and CRP levels before and following treatment were retrospectively examined from the patients' medical files. A decrease of 75% or more in ABSIS was considered disease improvement. The study was approved by Local Ethics Committee.

Statistical analysis

Statistical analyses were performed using SPSS version 22.0 (SPSS Inc, Chicago, IL, USA). The distribution of the compared values did not comply with the normal distribution according to the Kolmogorov-Smirnov test in patients with PV, and the Shapiro-Wilk test in patients with BP. The Wilcoxon Signed Ranks test was used for the comparison of MPV values,

thrombocyte, eosinophil and basophil counts, and ESR and CRP levels before and after treatment. A *P* value of <0.05 was considered significant.

RESULTS

Among patients with PV, 78 (40.8%) were men and 113 (59.2%) were women. The mean age at the onset of the disease was 52.53±14.94. The mean age at the onset of the disease and *P* values of inflammatory markers in patients with pemphigus during activation and remission are presented in Table 1.

Several inflammatory markers showed statistically significant decreases during the remission phase in patients with PV, including MPV value, eosinophil count, basophil count, and ESR and CRP levels. However, there was no statistically significant alteration in platelet count (Table 1).

Eighteen (39.1%) patients with BP were men and 28 (60.9%) were women. The mean age at diagnosis was 76.67±9.17. The mean, median, and *P* values of inflammatory markers in patients with BP during the activation and remission periods are presented in Table 2.

MPV values, eosinophil count, and ESR and CRP levels all showed statistically significant decreases during the remission phase in patients with BP, while platelet and basophil counts showed no statistically significant changes (Table 2).

DISCUSSION

Autoimmune diseases occur when the immune system perceives a part of the body as a pathogen and reacts against it. Genetic, immunological, environmental, and psychological factors are thought to play a role in the pathogenesis of autoimmune diseases (12). PV and BP are two significant autoimmune diseases of the skin. It is believed that antibodies against desmoglein 1 and 3 in PV and against BP180 and BP230 antigens in BP cause bulla formation (13). In individuals with genetic predisposition, CD4 + T-cell activation occurs with the presentation of autoantigens by HLA class II molecules. It has been shown that T-lymphocytes release Th2-dependent cytokines and regulate B-cell activity in the formation of these antibodies (14). However, in some studies on pemphigus, steric inhibition of anti-desmoglein antibodies, which causes loss of desmoglein adhesion, was suggested to be not sufficient on its own to lead to the loss of keratinocyte adhesion, and additional intracellular signaling pathways are thought to play a role (2). Pathogenic mechanisms of BP autoantibodies comprise complement activation, recruitment of inflammatory cells, release of proteolytic enzymes, and direct impairment of the adhesive functions of autoantigens. Complement activation occurs with the binding of BP autoantibodies to the target. C3a and C5a, which occur by complement activation, stimulate neutrophil and eosinophil chemotaxis and mast cell degranulation. MMP-9 and elastase secreted by neutrophils and eosinophils in BP contribute to the loss of cell-matrix adhesion in the basement membrane by breaking down extracellular matrix proteins (15,16).

It is known that platelets are important in the development and modulation of the immune-inflammatory response, as well as that they have well-known roles in homeostasis and thrombotic events

Table 1. MPV values, thrombocyte, eosinophil and basophil counts, and ESR and CRP levels in patients with pemphigus vulgaris during disease activation and remission

	Activation period Mean ± SD median (min, max)	Remission period Mean ± SD median (min, max)	*P* value
MPV (fL)	8.54±1.37 8.2 (6.3, 12.4)	7.91±1.35 7.5 (5.7, 11.5)	<0.05
Thrombocyte (1000 /uL)	306.96±101.90 284 (112, 837)	299.55±96.30 293 (88, 866)	0.34
Eosinophil (1000 /uL)	0.21±0.26 0.14 (0.001, 1.90)	0.11±0.38 0.06 (0.001, 5.31)	<0.05
Basophil (1000 /uL)	0.03±0.02 0.03 (0.001, 0.15)	0.03±0.29 0.02 (0.001, 0.18)	<0.05
ESR (mm / hour)	30.69±21.88 24 (3, 97)	20.91±18.54 16 (1, 98)	<0.05
CRP (mg/L)	10.95±18.56 5.14 (1, 155)	4.78±10.67 1 (0.3, 111)	<0.05

CRP: C reactive protein; ESR: erythrocyte sedimentation rate; max: maximum, min: minimum; MPV: mean platelet volume; SD: standard deviation

(17). There have been studies examining the importance of platelets in inflammatory skin diseases with different pathogenic mechanisms such as atopic dermatitis, urticaria, and psoriasis due to their important roles in the immune-inflammatory process (9).

MPV is a marker commonly used to demonstrate platelet function, thought to reflect inflammation and the activity of inflammatory diseases (18). It has been determined that psoriasis, psoriatic arthritis, and Behçet's disease may be associated with high MPV values, whereas rheumatoid arthritis, inflammatory bowel diseases and FMF attacks may be associated with low MPV values (19-23). In this study, we found that MPV values decreased significantly in patients with PV and BP during disease remission, while platelet counts demonstrated no significant alterations. Kridin *et al.* reported that MPV levels were significantly lower in patients with PV compared with controls and that MPV levels did not change significantly based on disease activity, whereas the number of platelets decreased significantly (24). In our study, unlike Kridin *et al.*, MPV levels decreased significantly during the remission phase, but no significant difference was noted in platelet count. Similar to the results of our study, Lyakhovitsky *et al.* found that MPV levels significantly decreased in patients with pemphigus while the disease was in the remission period (25). Rifaioglu *et al.* showed that MPV values were significantly higher than in healthy controls, and the difference in platelet counts was not significant in their study comparing MPV values of 19 patients with BP and 22 healthy controls (26). Our findings were similar to those of Rifaioglu *et al.*, who found that MPV values significantly decreased in patients with BP during the healing of the lesions and platelet count did not alter significantly. Since MPV values were elevated, but platelet counts were not, it has been suggested platelets play a role in the pathogenesis of autoimmune bullous diseases.

When eosinophils are active, they secrete proteins in their granules such as major basic protein, eosinophil cationic protein, and eosinophil peroxidase, and this contributes to inflammation (27). Information about the role of eosinophils in the pathogenesis of PV is limited. The prominence of eosinophil infiltration was reported in the biopsy of two pregnant patients with PV (28). The relationship between BP and eosinophils is more pronounced. Eosinophils and lymphocytes constitute the dermal infiltrate seen in BP. Lin *et al.* demonstrated that eosinophils are required in the cellular link between the immunoglobulin E (IgE) autoantibody and skin blistering in the murine model of BP (29). In addition, eosinophils that activate interleukin-5 (IL-5) can cause the separation of the dermo-epidermal component (15). In our study, the eosinophil count was higher in both PV and BP during the active phase. We found a significant decrease in the eosinophil count during disease remission in patients with both PV and BP. Marzano *et al.* reported that there was a correlation between plasma eosinophil levels and disease activity in patients with BP (30). This suggests that eosinophils may contribute to autoinflammation in autoimmune bullous disease.

It is known that basophils play an active role in IgE-dependent IL-4 production (31). It is hypothesized that basophils may play a role in Th2 mediated initiation of immunity by providing an early source of

Table 2. MPV values, thrombocyte, eosinophil and basophil counts, and ESR and CRP levels in patients with bullous pemphigoid during activation and remission

	Activation period Mean ± SD median (min, max)	Remission period Mean ± SD median (min, max)	*P* value
MPV (fL)	7.97±0.91 7.9 (6.7, 11.3)	7.34±0.845 7.15 (6, 11.2)	<0.05
Thrombocyte (1000 / uL)	281.43±74.1 274.5 (129, 584)	275.02±80.77 266.5 (111, 461)	0.505
Eosinophil (1000 / uL)	0.78±1.47 0.34 (0.001, 8.52)	0.14±0.18 0.08 (0.001, 0.78)	<0.05
Basophil (1000 / uL)	0.04±0.08 0.02 (0.001, 0.60)	0.03±0.03 0.02 (0.001, 0.17)	0.554
ESR (mm / hour)	38.48±29.65 30 (2, 112)	25.78±18.85 21,5 (1, 74)	<0.05
CRP (mg/L)	25.12±26.64 13.05 (1, 116)	13.25±23.67 6.07 (1, 135)	<0.05

CRP: C reactive protein; ESR: erythrocyte sedimentation rate; max: maximum; min: minimum; MPV: mean platelet volume; SD: standard deviation

IL-4 for Th2 differentiation. Autoimmune diseases occur when mechanisms preventing the autoreactivity of both T- and B-cells fail to function. Generally, T-cell-mediated responses are associated with the Th1 subset of CD4 + T-cells, while antibody-mediated mechanisms are generally associated with Th2 responses (32). Ugajin *et al.* stated that, in patients with BP, basophils could contribute to the degradation of proteins in the epidermal basal membrane region and thus to the formation of subepidermal bullae (33). Therefore, basophils may have a role in the formation of autoantibodies in autoimmune bullous diseases. In our study, basophil count was significantly lower in patients with PV during the remission period. Although patients with BP also presented a decrease in mean basophil count while the disease was in the remission period, this difference was not statistically significant.

Alterations in plasma concentrations of acute-phase reactants are mostly due to changes in the production of these proteins in hepatocytes. Alterations in the levels of acute-phase proteins can occur in the presence of infection, trauma, surgery, burns, immune-mediated diseases, and malignant diseases. Among acute-phase proteins, the ones most commonly used are ESR and CRP. ESR is largely dependent on the plasma concentration of fibrinogen and reflects the indirect measurement of plasma acute-phase protein concentrations. Therefore, CRP can be considered to be more specific to inflammation (6). CRP may play a role in both humoral and cellular inflammation by activating the complement system (34). In our study, we observed a significant decrease in both ESR and CRP levels in patients with PV and BP during disease remission compared with the activation period. Studies reporting ESR and CRP level alterations in autoimmune bullous diseases are limited. Rifaioglu *et al.* found no significant differences in ESR and CRP levels in 19 patients with bullous pemphigoid compared with controls consisting of 22 patients (26). The difference in our findings may be due to the fact that patients in the study had severe conditions and were under systemic therapy, with lesions that did not regress with topical treatment. Therefore, autoinflammation was higher in those patients.

CONCLUSION

We found a correlation between a decrease in MPV values, eosinophil counts, and ESR and CRP levels and the healing of lesions in patients with PV and BP, whereas a decrease in basophil count was observed only in patients with PV who had decreased disease activity. Autoantibodies against epidermal/subepidermal components occur in autoimmune bullous diseases as a result of inflammation. Platelets, eosinophils, and basophils in patients with PV, and platelets and eosinophils in patients with BP, may contribute to this inflammation. The present study suggests that, with the discovery of additional pathways contributing to the pathogenesis of autoimmune bullous diseases, new therapeutic approaches may be developed to help manage challenging cases.

References:

1. Kershenovich R, Hodak E, Mimouni D. Diagnosis and classification of pemphigus and bullous pemphigoid. Autoimmun Rev. 2014;13:477-81.
2. Sardana K, Garg VK, Agarwal P. Is there an emergent need to modify the desmoglein compensation theory in pemphigus on the basis of Dsg ELISA data and alternative pathogenic mechanisms? Br J Dermatol. 2013;168:669-74.
3. Mao X, Nagler AR, Farber SA, Choi EJ, Jackson LH, Leiferman KM, *et al.* Autoimmunity to desmocollin 3 in pemphigus vulgaris. Am J Pathol. 2010;177:2724-30.
4. Ruocco V, Ruocco E, Lo Schiavo A, Brunetti G, Guerrera LP, Wolf R. Pemphigus: etiology, pathogenesis, and inducing or triggering factors: facts and controversies. Clin Dermatol. 2013;31:374-381.
5. Kridin K, Bergman R. Assessment of the prevalence of mucosal involvement in bullous pemphigoid. JAMA Dermatol. 2019;155:166-71.
6. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999;340:448-54.
7. Kasperska-Zajac A, Brzoza Z, Rogala B. Platelet function in cutaneous diseases. Platelets. 2008;19:317-21.
8. Leader A, Pereg D, Lishner M. Are platelet volume indices of clinical use? A multidisciplinary review. Ann Med. 2012;44:805-16.
9. Wang JG, Mahmud SA, Thompson JA, Geng JG, Key NS, Slungaard A. The principal eosinophil peroxidase product, HOSCN, is a uniquely potent phagocyte oxidant inducer of endothelial cell tissue factor activity: a potential mechanism for thrombosis in eosinophilic inflammatory states. Blood. 2006;107:558-65.
10. Sokol CL, Barton GM, Farr AG, Medzhitov R. A mechanism for the initiation of allergen-induced T helper type 2 responses. Nat Immunol. 2008;9:310-8.
11. Sebaratnam DF, Murrell DF. Objective scoring systems for disease activity in autoimmune bullous disease. Dermatol Clin. 2011;29:515-20.

12. Ruocco E, Wolf R, Ruocco V, Brunetti G, Romano F, Lo Schiavo A. Pemphigus: associations and management guidelines: facts and controversies. Clin Dermatol. 2013;31:382-90.
13. Hammers CM, Stanley JR. Recent advances in understanding pemphigus and bullous pemphigoid. J Invest Dermatol. 2020;140:733-41.
14. Veldman C, Eming R, Wolff-Franke S, Sonderstrup G, Kwok WW, Hertl M. Detection of low avidity desmoglein 3-reactive T cells in pemphigus vulgaris using HLA-DR beta 1*0402 tetramers. Clin Immunol. 2007;122:330-7.
15. Amber KT, Valdebran M, Kridin K, Grando SA. The role of eosinophils in bullous pemphigoid: a developing model of eosinophil pathogenicity in mucocutaneous disease. Front Med (Lausanne). 2018;5:201.
16. Lo Schiavo A, Ruocco E, Brancaccio G, Caccavale S, Ruocco V, Wolf R. Bullous pemphigoid: etiology, pathogenesis, and inducing factors: facts and controversies. Clin Dermatol. 2013;31:391-9.
17. Elzey BD, Tian J, Jensen RJ, Swanson AK, Lees JR, Lentz SR, *et al.* Platelet-mediated modulation of adaptive immunity. A communication link between innate and adaptive immune compartments. Immunity. 2003;19:9-19.
18. Gasparyan AY, Ayvazyan L, Mikhailidis DP, Kitas GD. Mean platelet volume: a link between thrombosis and inflammation? Curr Pharm Des. 2011;17:47-58.
19. Kisacik B, Tufan A, Kalyoncu U, Karadag O, Akdogan A, Ozturk MA, *et al.* Mean platelet volume (MPV) as an inflammatory marker in ankylosing spondylitis and rheumatoid arthritis. Joint Bone Spine. 2008;75:291-4.
20. Yüksel O, Helvaci K, Başar O, Köklü S, Caner S, Helvaci N, *et al.* An overlooked indicator of disease activity in ulcerative colitis: mean platelet volume. Platelets. 2009;20:277-81.
21. Canpolat F, Akpinar H, Eskioğlu F. Mean platelet volume in psoriasis and psoriatic arthritis. Clin Rheumatol. 2010;29:325-8.
22. Milovanovic M, Nilsson E, Järemo P. Relationships between platelets and inflammatory markers in rheumatoid arthritis. Clin Chim Acta. 2004;343:237-40.
23. Sahin S, Senel S, Ataseven H, Yalcin I. Does mean platelet volume influence the attack or attack-free period in the patients with Familial Mediterranean fever? Platelets. 2013;24:320-3.
24. Kridin K, Shihade W, Zelber-Sagi S. Mean Platelet Volume in pemphigus vulgaris. Angiology. 2018;69:303-7.
25. Lyakhovitsky A, Dascalu J, Drousiotis T, Barzilai A, Baum S. Hematological Inflammatory Markers in Patients with Pemphigus Vulgaris. Dermatology. 2021;20:1-9.
26. Rifaioglu EN, Sen BB, Ekiz Ö, Dogramaci AC. Mean platelet volume and eosinophilia relationship in patients with bullous pemphigoid. Platelets. 2014;25:264-7.
27. Long H, Zhang G, Wang L, Lu Q. Eosinophilic Skin Diseases: A Comprehensive Review. Clin Rev Allergy Immunol. 2016;50:189-213.
28. Küçükoğlu R, Sun GP, Kılıç S. Polycyclic annular presentation of pemphigus vulgaris with an eosinophil predominance in two pregnant patients. Dermatol Online J. 2018;24:13030/qt89x9z770.
29. Lin L, Hwang BJ, Culton DA, Li N, Burette S, Koller BH, *et al.* Eosinophils Mediate Tissue Injury in the Autoimmune Skin Disease Bullous Pemphigoid. J Invest Dermatol. 2018;138:1032-43.
30. Marzano AV, Tedeschi A, Spinelli D, Fanoni D, Crosti C, Cugno M. Coagulation activation in autoimmune bullous diseases. Clin Exp Immunol. 2009;158:31-6.
31. Wedemeyer J, Tsai M, Galli SJ. Roles of mast cells and basophils in innate and acquired immunity. Curr Opin Immunol. 2000;12:624-31.
32. Emmi L, Romagnani S. Role of Th1 and Th2 cells in autoimmunity. In: The Autoimmune Diseases, Rose NR, Mackay IR (Eds), Academic Press, San Diego 2006. pp. 83.
33. Ugajin T, Takahashi M, Miyagishi C, Takayama K, Yokozeki H. A case of bullous pemphigoid associated with infiltration and activation of basophils. Br J Dermatol. 2015;173:1095-8.
34. Volanakis JE. Acute phase proteins in rheumatic disease. In: Koopman WJ, ed. Arthritis and allied conditions: a textbook of rheumatology. 13th ed. Baltimore: Williams & Wilkins, 1997:505-14.