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Association between Immunofluorescence Pattern and Mucosal Involvement in Patients with Bullous Pemphigoid

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ABSTRACT Bullous pemphigoid is an acquired autoimmune subepidermal blistering disease which is associated with mucocutaneous lesions. The type and amount of autoantibody deposition may have a role in mucosal lesions. We studied the association between mucosal involvement and direct immunofluorescence pattern in cutaneous lesions of patients with bullous pemphigoid.

In this retrospective analytical cross-sectional study, we studied the demographic data, clinical presentations, and immunopathological findings of 69 patients with bullous pemphigoid admitted to our hospital 2008-2016. Patients were allocated into two groups on the basis of the mucosal involvement, and direct immunofluorescence patterns were evaluated. The data were analyzed using SPSS version18.

The mean age of patients was 70.9±14.97 (mean ± Standard Deviation) years old. In our study, 56.5% of patients were women. All patients showed deposition of IgG and C3 in the dermoepidermal junction, with different severity. Patients with mucosal involvement (40.6% of cases) had a more prominent deposition of IgG, IgA, and C3 at the dermoepidermal junction compared with patients without mucosal involvement, which represented a statistically significant difference ($P<0.05$). Logistic regression analysis showed that lower age, IgA, and C3 deposition ($P<0.05$) were associated with mucosal involvement.

Deposition of IgA and C3 (in addition to IgG) at the dermoepidermal junction seems to be a marker of mucosal involvement in patients with bullous pemphigoid. Attention to direct immunofluorescence pattern in patients with bullous pemphigoid may be helpful in prediction of mucosal involvement in these patients.

KEY WORDS: bullous pemphigoid, direct immunofluorescence, mucosal involvement

INTRODUCTION

Bullous pemphigoid (BP) is an acquired autoimmune subepidermal bullous disease. Prevalence of BP has been increased in recent years. It usually occurs at old ages and is associated with autoantibody production, especially IgG antibodies, against hemidesmosomal components (BP antigen) (1,2). Other immuno-

reactant depositions include IgM, IgA autoantibodies, and complement component 3 (C3). Cutaneous lesions usually present as urticarial papules, plaques, and inflammatory or non-inflammatory intact bullae (1). About one third of patients with BP have mucosal involvement (2). Some authors believe that the type and amount of autoantibody depositions, especially IgA antibodies, may have a role in mucosal lesions in these patients, but there are contradictory reports in this regard (3,4). We studied the association between mucosal involvement and direct immunofluorescence (DIF) pattern of cutaneous lesions in patients with BP. Our hypothesis was that mucosal erosions are correlated with a different variety of antibody depositions.

PATIENTS AND METHODS

This research project was a retrospective analytical cross-sectional study; we evaluated the demographic data (age and sex), smoking history, clinical presentations (localized or generalized cutaneous involvement), histopathological pattern (eosinophil-rich or not), and immunofluorescence pattern (type and amount of immunoreactant depositions) of 69 patients with BP admitted to Razi hospital, Guilan University of Medical Sciences over the past nine years (2008-2016). We reviewed their files in the archive of our hospital. All patients with a definite diagnosis of BP were allocated into two groups on the basis of oral mucosal involvement, after which the DIF patterns of their cutaneous lesions were evaluated. Severity of basement membrane deposits was evaluated with objective assessment of the pathologist, scored from 1+ (weakest) to 4+ (strongest). Our patients had no treatment with immunosuppressive agents prior to diagnosis.

No patient had mucosal biopsy and indirect immunofluorescence (IIF) study. Diagnosis of BP was established on the basis of clinical examination and DIF study of the skin lesions. The obligatory criterion for diagnosis was linear deposition of IgG and C3 along the basement membrane on DIF study. Major criteria included: 1) Tense blisters and/or erosions on the skin and rarely mucosa; 2) Histopathological findings of subepidermal blisters associated with eosinophil infiltration (1). Minor criteria were not used for diagnosis in this study because immunoblotting, enzyme-linked immunosorbent assay (ELISA), and IIF techniques were not available in our hospital, so the definite diagnosis of BP in our study was based on the obligatory criterion plus two major criteria. We used SPSS 18 software to analyze the data. The quantitative data from this study were examined and presented as mean and standard deviation (SD). The qualitative data from this study were described by frequency and percentage. The relationships between different variables and mucosal involvement were analyzed using the Chi-square test, Mann-Whitney test, and logistic regression. A logistic regression model was used to assess the associations between effective factors and mucosal involvement. In the process of model building, variables with a *P*-value <0.2 in the univariate analysis were first entered into the multiple regression model. The association between each variable and mucosal involvement in the multiple regression

Table 1. Characteristics of basement membrane depositions in patients with bullous pemphigoid with and without mucosal involvement

Basement membrane depositions		Patients without mucosal involvement Number (%)	Patients with mucosal involvement Number (%)	total Number (%)	P-value*
IgG	1+	19 (46.34)	6 (21.43)	25 (36.23)	0.029
	2+	17 (41.46)	12 (42.86)	29 (42.03)	
	3+	5 (12.20)	10 (35.71)	15 (21.74)	
C3	1+	15 (36.59)	3 (10.71)	18 (26.09)	0.034
	2+	16 (39.02)	12 (42.86)	28 (40.58)	
	3+	10 (24.39)	13 (46.43)	23 (33.33)	
IgM	0	28 (68.29)	17 (60.71)	45 (65.22)	0.516
	1+ or 2+	13 (31.71)	11 (39.29)	24 (34.78)	
IgA	0	28 (68.29)	10 (35.71)	38 (55.07)	0.008
	1+ or 2+	13 (31.71)	18 (64.29)	31 (44.93)	

**P*-values were calculated using Chi-Square test

model was expressed as an odds ratio (OR) with 95% confidence intervals (CIs). All *P*-values were two sided; significance level was set at $P<0.05$.

This research project was approved by the Ethics committee of Guilan University of Medical Sciences (Code: IR.GUMS.REC.1395.206).

RESULTS

Sixty nine patients with BP were enrolled in this study. The mean age of patients was 70.9±14.97 years old with a variation range of 24-95 years. Most of the patients were aged between 70 to 90 years. Two out of 69 patients were less than 40 years old: one was 24 years old and had severe congenital skeletal abnormality, and another was 38 years old.

More than half of patients (56.5% of cases) were women, and 43.5% of cases were men. More than one third of patients (40.6% of cases) had mucosal involvement, and there was no significant association between mucosal involvement and sex ($P=0.683$). Mean age in the group with mucosal involvement was 66.85 years, versus 73.66 years in the group without mucosal involvement ($P=0.052$).

Characteristics of basement membrane depositions in patients with BP with and without mucosal involvement are presented in Table 1. All patients showed depositions of IgG and C3 in the dermoepidermal junction, with different severity on DIF studies. There was no IgM deposition in 65.2% of patients, but 33.3% of cases had 1+ and 1.4% of cases had 2+ IgM deposition. In more than half of patients, no IgA deposition was detected (55.1% of cases), while 1+ IgA deposition and 2+ IgA deposition were found in in 40.6% and 4.3% of cases, respectively.

Table 2. The final model of the adjusted ORs for effective factors on the mucosal involvement using logistic regression

variable	OR	95% CI	*P*-value
age	0.95	0.91, 0.99	0.013
IgA			
no	1.00		
yes (1+ or 2+)	4.56	1.31, 15.96	0.017
IgG			
+1	1.00		
+2	1.16	0.29, 4.62	0.830
+3	2.91	0.45, 18.73	0.261
C3			
+1	1.00		
+2	5.68	1.00, 32.19	0.050
+3	8.40	1.31, 53.84	0.025

OR: Odds Ratio; CI: Confidence Interval

There was no significant association between IgM deposition and mucosal involvement in patients with BP ($P=0.516$), but there was a significant difference in IgG, IgA, and C3 depositions at the dermoepidermal junction between the two groups ($P=0.029$, $P=0.008$, and $P=0.034$, respectively).

Other information obtained from our data was as follows: 87% of cases were non-smokers, 24.6% of cases had localized lesions on acral areas, and 5.8% of tissue samples were not eosinophil-rich. We did not find any significant association between mucosal involvement and smoking history, extent of the skin lesions (localized or generalized type), and pathologic patterns (eosinophil-rich or not). Mucosal involvement in our patients was mainly detected on oral examination where intact or hemorrhagic bullae, erosion, and desquamation were seen on the hard palate and buccal and gingival mucosa. Only four patients had mucosal involvement on genital and nasal areas in addition to oral lesions. The most common locations of mucosal involvement were the hard palate and buccal area, which were involved in 100% and 71.4% of cases with mucosal lesions, respectively. Tzanck smear evaluation of mucosal lesions was also performed for some patients and was negative for Tzanck cells.

According to multiple logistic regression analysis, the adjusted OR for mucosal involvement associated with age was 0.95 (95% CI, 0.91 to 0.99), which indicates that chance of mucosal involvement decreased about 5% per each one-year increase in age. IgA deposition increased the OR of mucosal involvement by 4.56 (95% CI, 1.31 to 15.96) compared with cases without IgA deposition. Patients with 2+ C3 deposition were 5.68 times more likely to have mucosal involvement compared with patients with 1+ C3 deposition (95% CI, 1.00 to 32.19). Furthermore, chance of mucosal involvement in 3+ C3 deposition subjects was higher than in cases with 1+ C3 deposition (OR=8.40, 95% CI, 1.31 to 53.84) (Table 2).

DISCUSSION

BP has been described as a common type of pruritic immunobullous disease which is more prevalent in the elderly (2). Mean age of patients in our study was lower than reported by Kirtschig *et al.* and more than in studies by Laskaris, Banihashemi, and Esmaili *et al.* (5-7). We performed a search on BP in young patients because we found two patients aged less than 40. Banihashemi *et al.* and Esmaili *et al.*

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described age ranges of 19-115 years and 12-100 years in their reports (2,7). The exact cause of increase in BP incidence after the sixth decade of life is unknown. Drug intake such as diuretics, analgesics, antibiotics, anti-hypertensives, and neuroleptic agents has been incriminated as a triggering factor in inducing BP. Taking such drugs usually increases in old age, but genetic predisposition is also of great importance (1). Chronic exposure to ultraviolet radiation and underlying skin diseases could expose basement membrane autoantigens to the immune system, which results in autoimmune bullous diseases in elderly patients (8). On the other hand, neurologic disorders have been significantly associated with BP in the elderly. The probable etiology for this association could be presentation of the neuronal form of BP antigen 230 in the central nervous system (1).

Prior studies have provided contradicting reports on sex predominance in BP, with some reporting female predominance and some reporting male (1,2,7). Our study found more frequent involvement in women.

Mucosal involvement was detected in more than one third of patients in our study, especially on the palate and buccal mucosa, but it has been reported with different rates in other studies. Mucosal erosions have been found in 10% to 45% of patients with BP, usually occurring on the palate, buccal mucosa, tongue, gingiva, and lips, but other mucosal surfaces including genital, nasal, anal, or rarely conjunctival mucosae may be involved (7-12). Interestingly, Laskaris *et al.* reported subclinical mucosal involvement in the form of antibody depositions on the basement membrane in 80% of patients with BP (6).

In our study, all patients showed IgG and C3 depositions and some had IgM and IgA depositions on the basement membrane in cutaneous lesions; unfortunately we had no mucosal biopsy available.

BP antigens are easily diagnosed and targeted by different autoantibodies and complements including IgG, IgM, IgA, IgE, and C3 (8). About one third of our cases had IgM deposition, and 44.9% of cases had IgA deposition, which is more than prior studies that reported 20% and 28% of patients with BP had IgA or IgM autoantibodies, respectively (3,13). Christophoridis *et al.* explained that in severe forms of BP, including patients with oral involvement, the IIF method was indicative of both IgG and IgA production in the serum of patients (4); unfortunately, the IIF method was not performed in our patients.

Maurice *et al.* found that half of patients with BP with IgA autoantibody had mucosal involvement and these cases had more severe disease with delayed remission and more recurrences (13). In our study, about two third of patients with mucosal involvement had IgA deposition, while about one third of cases without mucosal involvement had IgA deposition.

Our study showed that IgA production raises the chance of mucosal involvement 4.5 times. Also severity of C3 deposition had a role in mucosal erosions. However, the pathogenic role of IgA in development of mucosal lesions has not been established, and there are contradictory reports in this regard (4,5,14). It has been shown in animal models that IgA deposition may be a trigger for neutrophil chemotaxis, which results in basement membrane destruction and mucosal erosions in patients with BP (14); so adjuvants with anti-inflammatory effects may be needed in these cases. Generally, it seems the presence of secondary antibodies in addition to IgG is indicative of disease severity, and the remission rate in these cases has also been low while the recurrence rate during the maintenance phase has been high (12,16). Zhou *et al.* reported that there was a correlation between mucosal involvement and disease severity (17). Iwata *et al.* showed that there was a correlation between the level of IgE antibodies against BP antigen 2 and severity of activity of the disease (18). IgE detection was not performed for our cases.

A new data-point not present in other studies was the lower incidence of mucosal lesions at older ages, as a one-year increase in age decreased the chance of mucosal involvement by about 5%. This finding should be reevaluated in other studies with larger sample size. We think that BP antigen presentation in mucosal membranes may be decreased in the elderly, which should be investigated in future.

We did not have a meticulous scoring system for evaluation of disease severity in our patients, but there was no significant difference in mucosal involvement between localized and generalized disease. Unfortunately, we missed some patients with localized BP because such cases were not referred to our hospital; consequently, this result was not reliable.

We did not find any correlation between smoking and mucosal involvement, which could be explained by low frequency of smoking in our study. It has been shown that smoking has a protective effect against mucosal erosions in patients with pemphigus vulgaris (19).

Linear IgA/IgG dermatitis (LAGBD) is a new entity which could mimic BP and should be evaluated further in the future (20,21). These patients have reactive IgA to BP antigen 2 and generalized pruritic bullae, but despite our hypothesis there is no significant mucosal involvement in LAGBD patients (20-23).

Immunoblotting method suggests a different variety of targeted antigens in LAGBD (23), but unfortunately this technique was not available to us. With the lack of accurate techniques and also considerable overlapping features between BP, LAGBD, and linear IgA bullous dermatosis, clinical judgment in these cases might be questionable.

Mucosal involvement is currently not a usual finding in LAGBD; however, in our opinion, BP with IgA deposition could be reclassified as LAGBD with prominent mucosal lesions.

About 25-30% of patients with mucous membrane pemphigoides (MMP) may also have skin lesions, but mucosal involvement in MMP is usually associated with scar formation or occasionally adhesion bands and ectropion during disease progression. Skin involvement could also be associated with milia formation (1). In our patients, scar formation, adhesion bands, or milia were not detected during 6 months follow-up. All patients were totally controlled with oral corticosteroids, so MMP could not be considered because systemic corticosteroids alone are usually insufficient for MMP (1).

Our limitations in this retrospective study were small sample size, not performing mucosal biopsy for patients with BP, lack of a scoring system for accurate evaluation of skin and mucosal involvement, no admission for localized BP, and no availability of IIF study, immunoblotting, ELISA, and IgE detecting techniques in our center.

In analyzing our final results using a logistic regression model, it seems that some factors other than the variables which were included in our study may also have a role in mucosal involvement in patients with BP. These factors include Koebner's phenomenon due to denture trauma, candida infection, low oral or dental hygiene, micronutrient deficiencies, and the patient's diet or drug consumption.

CONCLUSION

We conclude that IgA and C3 deposition at the dermoepidermal junction seems to be a marker of mucosal involvement in patients with BP. Attention to the direct immunofluorescence pattern in these patients may be helpful in treatment planning and prediction of mucosal involvement.

More studies using a larger sample size, accurate scoring system for evaluation of skin and mucosal involvement, IIF study, immunoblotting, ELISA, and IgE detecting techniques are recommended for more accurate results. Additionally, the role of disease duration in mucosal involvement should be investigated in future studies.

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