

A Cross-Sectional Study to Correlate Disease Severity in Bullous Pemphigoid Patients with Serum Levels of Autoantibodies Against BP180 and BP230

Abstract

Context: Enzyme-linked immunosorbent assay (ELISA) for BP 180 and 230 antibodies is commonly done in patients with bullous pemphigoid. We could not find much data regarding the usefulness of this test to predict the disease severity in Indian population. **Aims:** We studied the correlation of IgG anti BP180 and anti BP230 antibody titer with disease severity and clinical features in bullous pemphigoid. **Settings and Design:** This cross-sectional study was conducted at a tertiary care center in western India. **Materials and Methods:** Forty-two clinically diagnosed treatment-naive cases of bullous pemphigoid were enrolled and investigated with skin punch biopsy, IgG anti BP180, and anti BP230 ELISA, direct immunofluorescence, and indirect immunofluorescence tests. Disease severity was assessed by calculating modified Autoimmune Bullous Skin Disorder Intensity Score (ABSIS) score. Thirty patients with a final diagnosis of bullous pemphigoid were included in the statistical analysis. Pearson's correlation coefficient (r) was used to study correlation. **Results:** The mean ABSIS skin score was 32.81 when both tests were negative, 42.13 when only BP230 was positive, 76.28 when only BP180 was positive, and 78.16 when both were positive. Pearson's correlation coefficient (r) for BP180 and ABSIS skin score was 0.6 (P value: 0.0005), and for BP230 was -0.055 (P value: 0.600). **Conclusions:** BP antibody titers correlate partially with disease severity. Anti-BP180 antibody is associated with more severe disease. Anti-BP230 antibody titer does not correlate with disease severity.

Keywords: Anti-BP180 NC16A, Anti-BP230, autoimmune bullous skin disorder intensity score, bullous pemphigoid

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Introduction

Bullous pemphigoid (BP) is an autoimmune blistering disorder in which autoantibodies against hemidesmosomal proteins BP180 and BP230 evoke subepidermal blister formation. It is primarily a disease of the elderly. The presence of antibodies in serum with titer can be demonstrated by Enzyme-linked immunosorbent assay (ELISA) test. Various studies have proved the correlation of antiBP180 antibody ELISA titer with disease severity.

Bullous pemphigoid is the second most common immunobullous disease in India.^[1,2] This study was an attempt to fill the lacunae in our knowledge regarding the prevalence of clinical features and correlation of antibody titers with clinical features and disease severity in Indian population.

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Materials and Methods

This cross-sectional study was initiated after obtaining permission from the institutional ethics committee at a tertiary care center in Western India. The study duration was 18 months. Forty-two clinically diagnosed cases of bullous pemphigoid were enrolled. The convenience sampling method was followed. The newly diagnosed but treatment-naive patients as well as previously diagnosed cases, who were off treatment for at least 6 weeks were included in this study. BP was diagnosed based on clinical features, histopathological examination, and immunofluorescence studies. When all three were suggestive of BP, patients were included in the analysis irrespective of BP180 and 230 ELISA test results. The patient selection process is detailed in Figure 1.

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The disease severity was scored using a modified Autoimmune Bullous Skin Disorder Intensity Score (ABSIS) skin score. Extensive mucosal involvement is not a feature of BP. We did not consider mucosal score in the analysis as the contribution of mucosal score to the disease was totally absent or negligibly small in our patients. Boulard C, *et al.*^[3] defined cut off values of ABSIS score as 17 and 53 to define moderate, extensive and significant disease in pemphigus vulgaris by calculating 25th and 75th score percentiles. Cut-off values for the severity of bullous pemphigoid are not yet defined. Excluding the mucosal score, we arbitrarily defined the cut-off score as 12 and 45 for mild, moderate and severe bullous pemphigoid, respectively.

Quantitative assessment of IgG antibodies against BP180NC16A and BP230 was done using ELISA kit (Euroimmun, Lubeck, Germany) in all patients during the same visit. 20 RU/mL was the cut-off titer to define negative and positive tests. This diagnostic kit had a limit range of up to 200 RU/mL. Results were not satisfactory when the serum samples were diluted up to 1:1000. So, the titers below 2 as well as above 200 were considered as 2 and 200, respectively, during statistical analysis.

Direct immunofluorescence was done in 37 patients, whereas indirect immunofluorescence was done in 5 patients where direct immunofluorescence was inconclusive. Histopathological examination was done in 33 patients.

Twelve patients were excluded from the analysis because of alternate diagnosis or failure in obtaining complete standard investigation reports. There were two cases of pemphigus vulgaris, one epidermolysis bullosa aquisita, one bullous lichen planus, and one paraneoplastic pemphigus. One patient was diagnosed with dermatitis herpetiformis/linear IgA disease. Direct immunofluorescence test was not done in this patient due to lack of resources. Patients with paraneoplastic pemphigus and dermatitis herpetiformis/linear IgA disease showed positive test for anti BP180 antibody. Three patients were not willing to do either ELISA or direct

immunofluorescence test. One patient presented with tense bullae on an urticarial base with a history of renal cell carcinoma showed no evidence of bullous pemphigoid in any investigation. One patient was diagnosed with irritant contact dermatitis with autosensitization. ELISA for anti-BP180 was positive, and direct immunofluorescence was negative in this patient.

Thirty patients with a final diagnosis of BP were included in statistical analysis. Frequencies of categorical variables were presented as proportion. Quantitative variables were presented in terms of mean, standard deviation, and range. Pearson's correlation coefficient was used to correlate disease severity score and clinical features with antibody titer. $P < 0.05$ was considered statistically significant. SPSS software was used for statistical analysis.

Results

The mean age of patients was 56.33 ± 13.69 years with a range of 18–79 years. Female preponderance was observed with 13 (43.33%) male and 17 (56.67%) female patients. The mean duration of disease was 8.3 ± 7.31 months with a range of 0.1–24 months.

The prevalence of various clinical features is depicted in Figure 2. Oral mucosa was involved in 46.67% in the form of erosion or vesicle. All of them had a score of 1 despite the highest possible objective mucosal score of 11. The lesions did not interfere with food intake in any of them.

BP180 antibody was present in 16 (53.33%) patients, and BP230 antibody was present in 14 (46.67%) patients. Eight patients (26.67%) had both tests negative. The prevalence of various antibodies and the mean disease severity score in these patients are given in Table 1.

The mean titer of antiBP180 and antiBP230 antibodies were 91.03 ± 89.45 and 45.5 ± 64.3 , respectively with a range of 2–200. The mean disease severity score was 58.36 ± 38.84 with a range of 1.5–130.5.

Correlation of disease severity (ABSIS skin score) with antiBP180 and antiBP230 antibody titer was checked using Pearson's correlation coefficient (r). r value for

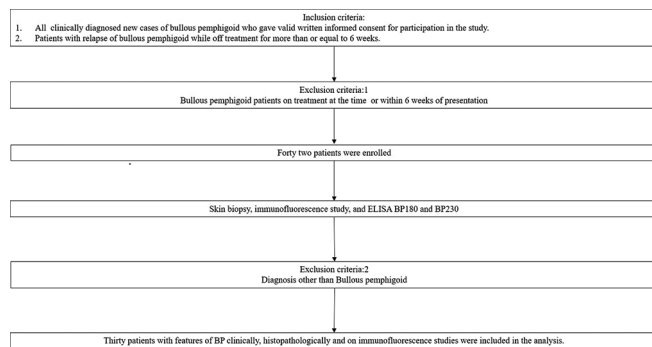


Figure 1: Flow chart depicting patient selection process of this cross-sectional study

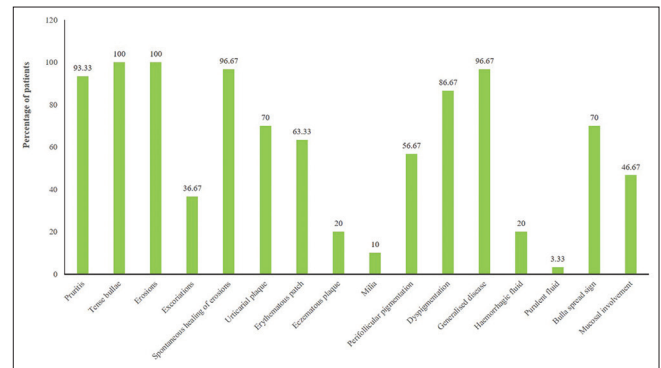


Figure 2: Prevalence of various clinical manifestations in bullous pemphigoid patients (n = 30)

BP180 was 0.6 (*P* value: 0.0005). *r* value for BP230 was -0.055 (*P* value: 0.600).

The correlation of clinical features of bullous pemphigoid with antiBP180 and antiBP230 antibody titer was checked using Pearson’s correlation coefficient (*r*). Perifollicular pigmentation showed statistically significant correlation with antiBP180 antibody titer (*r* = 0.396, *P* = .031). No other clinical feature showed such a correlation [Table 2].

Discussion

In Western populations, BP commonly occurs during the 7th decade or thereafter. De D, *et al.*^[1] have reported the mean age of BP in Indian population as 59 years. In our study, the mean age was 56.33 ± 13.69 years. The reason for occurrence of BP at an earlier age in the Indian population is not known. The sex ratio showed a slight female preponderance with 56.67% females which is consistent with most of the studies conducted in European and Asian populations. The female to male ratio varies between 0.64 and 5.1 in different populations across the globe.^[4]

Clinical features

As other Indian studies were not mentioning clinical features in detail, the comparison was drawn between the current study and other Asian studies in Table 3.

Bourdon-Lanoy E, *et al.*^[7] observed more severe disease in a group of young bullous pemphigoid patients with a mean age of 46 ± 11.6. We observed a lower mean age of 56.33 ± 13.69 years in Indian patients as compared to Thai and Iranian patients. The current study finding of higher prevalence of clinical features such as pruritus, erosions, and urticarial plaques indicates the higher severity of BP in Indian population who tends to get affected at an earlier age. These findings are in agreement with the conclusion of Bourdon-Lanoy E, *et al.*^[7]

Clapé A, *et al.*^[8] have reported the association of mucosal involvement with increased severity of BP. The higher prevalence of mucosal involvement in Indian population with higher prevalence of above mentioned clinical features suggest relatively severe nature of BP in Indian population.

Clinical variation according to antibody profile and titer

We observed variation in disease severity according to the number and types of antibodies present in an individual. The coexistence of both antibodies led to the most severe disease and hence they required more aggressive management. The least disease severity was observed when both the antibodies were negative. Such patients required less aggressive treatment. Disease severity was more in patients with isolated BP180 antibody positivity as compared to patients with isolated BP230 antibody positivity (anti-BP230 type bullous pemphigoid).

Table 1: Mean ABSIS skin score in groups of bullous pemphigoid patients with various antibody combinations (n=30)

Test result	Number	%	Mean ABSIS skin score
Both Bp180 and 230 Negative	8	26.67%	32.81
Only BP230 positive	6	20.00%	42.13
Only BP180 positive	8	26.67%	76.28
Both Bp180 and 230 Positive	8	26.67%	78.16

ABSIS - Autoimmune Bullous Skin Disorder Intensity Score

Table 2: Correlation of antiBP180 and antiBP230 antibody titer with clinical features of bullous pemphigoid (n=30)

Clinical features	BPAg180		BPAg230	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Pruritus	0.286	0.126	0.250	0.183
Excoriations	0.018	0.923	-0.157	0.407
Spontaneous healing of erosions	0.199	0.293	0.174	0.359
Urticarial plaque	0.262	0.161	-0.117	0.539
Erythematous patch	0.259	0.167	0.157	0.407
Eczematous plaque	-0.033	0.861	-0.301	0.106
Milia	-0.134	0.481	0.134	0.481
Perifollicular pigmentation	0.396	0.031	0.009	0.962
Dyspigmentation	0.026	0.891	-0.223	0.237
Fresh episode	-0.111	0.560	-0.040	0.833
Generalized disease	0.199	0.293	0.174	0.359
Hemorrhagic fluid	0.301	0.106	-0.134	0.481
Purulent fluid	0.174	0.359	-0.174	0.359
Mucosal involvement	0.205	0.276	0.063	0.743

Hayakawa T, *et al.*^[9] proposed the concept of anti-BP230 type bullous pemphigoid in 2016. Antibody against BP230 is the only antibody detected in this group. BP230 antibodies are considered pathogenic in this variant of BP. Epitope spreading is the mechanism of BP230 antibody development in BP patients with BP180 and BP230. The mechanism of development of antibodies is different in anti-BP230 type bullous pemphigoid. This variant shows less severity and requires fewer systemic steroids. The current study also showed lesser severity in patients with isolated BP230 positivity.

Van Beek N, *et al.*^[10] studied the correlation of BP180 IgG and IgE antibodies with clinical features. IgG anti BP180 NC16A antibodies showed association with erosions and blisters but not with urticarial and erythematous lesions. IgE anti BP180 NC16A antibodies did not show any correlation with erosions or blisters or urticarial and erythematous lesions. The current study did not show any statistically significant correlation between IgG anti-BP180 NC16A antibodies and erosions or blisters. Perifollicular pigmentation showed positive correlation with anti BP180 antibodies (*r* = 0.396, *P* = 0.031). This correlation does not have any clinical significance in patient management.

Clapé A, *et al.*^[8] reported the absence of BP230 antibody in patients with mucosal involvement. We could not find such an association in the current study. None of the clinical features correlated with BP230 antibodies.

Variation in disease severity according to antibody titer

Pathogenic antibodies against bullous pemphigoid antigens can be of class IgG or IgA or IgE. IgG antibodies may belong to IgG1 or IgG3 or IgG4 subclass. IgE and IgG targeting BP180 are known for their correlation with disease severity in bullous pemphigoid. We studied class IgG antibodies against BP180 and BP230. As Indian studies were not available, the comparison was drawn between the current study and other available studies in Tables 4 and 5.

The aim of the current study was to look for a correlation between IgG antibodies against BP180 and BP230, and

disease severity. Anti BP180 IgG antibody titer showed positive correlation with disease severity score ($r = 0.6$, $P = 0.0005$). This shows a statistically significant but weak correlation. Many authors have noted a correlation between AntiBP180 antibody titer and disease severity like the current study.^[11-16] The consistent correlation of BP180 antibody to disease severity in different populations is in agreement with the school of thought that BP180 antibody has got a definite role in the pathogenesis of BP.

Anti BP 230 IgG antibody titer did not show any statistically significant correlation with ABSIS skin score in our study. Lee EH, *et al.*,^[12] Patsatsi A, *et al.*^[16], and Daneshpazhooh M, *et al.*^[11] also observed the absence of correlation between anti BP230 titer and disease severity.

Mean ABSIS score was less in patients with only BP230 antibodies where BP230 is considered as pathogenic. This

Table 3: Prevalence of clinical features of bullous pemphigoid in different populations

Features	Current study (%)	Kulthanan K, <i>et al.</i> ^[5] (%)	Banihashemi M, <i>et al.</i> ^[6] (%)
Country	India	Thailand	Iran
Mean age	56.33±13.69 years	69±14.7 years	69 years
Mucosal involvement	46.67	12 (at onset) 15.5 (during clinical course)	3.2
Pruritus	93.33	79.3	84.3
Tense bullae	100	100	27.3 (on admission) 100% (during course of disease)
Erosions	100	56.9	-
Urticarial plaque	70	39.7	36.8
Erythematous patch	63.33	-	-
Eczematous plaque	20	-	32.7

Table 4: Correlation of BP antibody ELISA titers with disease severity in different populations

Reference	Sample size	Result		ELISA kit used
		Correlation between BP180 ELISA titer and disease activity	Correlation between BP230 ELISA titer and disease activity	
Daneshpazhooh M, <i>et al.</i> ^[11]	95	Present	Absent	Euroimmun, L€ubeck, Germany
Current study	30	Present	Absent	Euroimmun, L€ubeck, Germany
Lee EH, <i>et al.</i> ^[12]	47	Present	Absent	MBL Co. Ltd, Japan
Cai SC, <i>et al.</i> ^[13]	34	Present	-	MBL Co. Ltd, Japan
Tsuji-Abe Y, <i>et al.</i> ^[14]	14	Present	-	-
Feng S, <i>et al.</i> ^[15]	20	Present	-	MBL Co. Ltd, Japan
Patsatsi A, <i>et al.</i> ^[16]	39	Present	Absent	MBL Co. Ltd, Japan

BP - bullous pemphigoid; ELISA - enzyme linked immunosorbent assay

Table 5: Anti BP180 and BP230 antibody profile in different populations

Reference	Antibody profile					
	BP180 antibody positive (%)	BP230 antibody positive (%)	Only BP180 antibody positive (%)	Only BP230 antibody positive (%)	BP180 and BP230 antibodies positive (%)	BP180 and BP230 antibodies negative (%)
Current study	53.33	46.67	26.7	20	26.7	26.7
Lee EH, <i>et al.</i> ^[12]	97.9	72.3	27.7	2.1	70.2	0
Tsuji-Abe Y, <i>et al.</i> ^[14]	78	-	-	-	-	-
Feng S, <i>et al.</i> ^[15]	95	-	-	-	-	-
Patsatsi A, <i>et al.</i> ^[16]	100	66	-	-	-	-

variant is known to manifest with less severity, and hence lower requirement of systemic steroids.^[9] This observation might be an indication of the correlation between disease severity and BP230 in those patients where BP230 antibody is pathogenic. But the current study as well as other similar studies reported an absence of correlation between disease severity and BP230 antibodies. This may be true for patients with both the antibodies where BP230 is considered nonpathogenic. But none of the studies checked for such a correlation in BP230 type bullous pemphigoid alone. So, further separate studies are required for BP230 type bullous pemphigoid and BP with both BP180 and BP230 to look for a correlation between BP230 antibody titer and disease severity.

There is limited data regarding the positivity rate of BP180/230 in Indian population. The current study shows a low positivity rate of BP 180/230 in the Indian population as compared to previous reports [Table 5]. This may be due to differences in the populations studied. Most of the available studies have used MBL. Co. Ltd, Japan ELISA kit. Our study was conducted using Euroimmun, L€ubeck, Germany ELISA kit. Daneshpazhooh M, *et al.*^[11] also used the same kit, but the study does not mention the positivity rate of individual antibodies [Table 4].

The correlation between BP180 antibody titer and the disease severity extends the scope for further research to study the correlation of antibody titer with treatment response.

Limitations

Small sample size and cross-sectional study design were the limitations of this study. As BP 180/230 titers are dynamic, more appropriate study design would be longitudinal with ELISA estimation at regular intervals. As the total duration available for the study was 18 months, and also due to the lack of resources for repeated ELISA testing, cross-sectional study design was selected. ABSIS skin scoring system does not have the provision to assess the severity of urticarial/erythematous lesions. Bullous Pemphigoid Disease Area Index (BPDAI) scoring system would be a better tool as it has got superior validity, reliability, and provision to score different subcomponents as compared to ABSIS score. Cut-off values to define severe disease were defined arbitrarily. 25th and 75th percentiles of ABSIS skin score were not calculated. The mucosal score was not considered while calculating the disease severity score. Correlation of disease severity with subclasses of IgG and other classes of antibodies such as IgE were not assessed in our study.

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Conflicts of interest

There are no conflicts of interest.

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