Clinical, demographic and immunopathological spectrum of subepidermal autoimmune bullous diseases at a tertiary center: A 1-year audit

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ABSTRACT

Background: The subepidermal autoimmune bullous diseases are a subset of immunobullous diseases encountered less frequently in the Indian population. There is a paucity of data on the prevalence, demographic and clinicopathological spectrum of various subepidermal autoimmune bullous diseases from India. Aim: To determine the demographic and clinicopathological profile of subepidermal autoimmune bullous diseases in Indian patients, presenting to the Immunobullous Disease Clinic of Postgraduate Institute of Medical Education and Research, Chandigarh. Methods: Patients seen from November 2013 to November 2014 who fulfilled the preset diagnostic criteria of subepidermal autoimmune bullous diseases were identified from case records. Data regarding demographic characteristics, clinical profile, immunopathological findings and treatment were collected from the predesigned proforma. Results: Of 268 cases of autoimmune bullous diseases registered, 50 (18.7%) were subepidermal autoimmune bullous diseases. Bullous pemphigoid was most frequently seen in 20 (40%) cases, followed by dermatitis herpetiformis in 14 (28%), mucous membrane pemphigoid in 6 (12%), chronic bullous dermatosis of childhood / linear immunoglobulin A bullous dermatosis in 5 (10%), lichen planus pemphigoides in 3 (6%), pemphigoid gestationis and epidermolysis bullosa acquisita in 1 (2%) case each. None of the patients had bullous systemic lupus erythematosus. Limitations: We could not perform direct and indirect immunofluorescence using salt-split skin as a substrate and immunoblotting due to non-availability of these facilities. Therefore, misclassification of subepidermal autoimmune bullous diseases in some cases cannot be confidently excluded. Conclusion: Subepidermal autoimmune bullous diseases are not uncommon in Indian patients. Bullous pemphigoid contributes maximally to the burden of subepidermal autoimmune bullous diseases in India, similar to that in the West, although the proportion is lower and disease onset is earlier. Dermatitis herpetiformis was observed to have a higher prevalence in our population, compared to that in the West and the Far East countries. The prevalence of other subepidermal autoimmune bullous diseases is relatively low. Detailed immunofluorescence and immunoblotting studies on larger patient numbers would help better characterize the pattern of subepidermal autoimmune bullous diseases and their features in Indian patients.

Key words: Bullous pemphigoid, demographic, dermatitis herpetiformis, India, subepidermal autoimmune bullous diseases

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INTRODUCTION

Autoimmune bullous diseases are broadly categorized into intraepidermal and subepidermal. Subepidermal autoimmune bullous diseases largely comprise of bullous pemphigoid, lichen planus pemphigoides, mucous membrane pemphigoid, pemphigoid gestationis, chronic bullous dermatosis of childhood / linear immunoglobulin A bullous dermatosis, dermatitis herpetiformis, epidermolysis bullosa acquisita and bullous systemic lupus erythematosus. They are considered to be infrequent in India compared to the West. The data regarding the demographics and characteristics of subepidermal autoimmune bullous diseases in India are limited to a few case reports and a limited number of studies on a small number of patients. This study tries to provide preliminary data on the clinical, demographic and immunopathological profile of various subepidermal autoimmune bullous diseases as seen over a year in a clinic setting.

METHODS

The present study is an audit of patients with subepidermal autoimmune bullous diseases registered in the Immunobullous Disease Clinic of Postgraduate Institute of Medical Education and Research, Chandigarh seen over a period of 1 year (November 2013 to November 2014). Patients were diagnosed based on a combination of clinical, histopathological and direct immunofluorescence findings according to the criteria detailed for each subepidermal autoimmune bullous disease in Table 1. Demographic details, clinical presentation, associated systemic diseases, histopathology, direct immunofluorescence findings and treatment administered were retrieved from the predesigned proforma of the Immunobullous Disease Clinic. Clinically suspected cases of subepidermal autoimmune bullous diseases with non-specific histopathology and/or negative direct immunofluorescence results were labeled as indeterminate.

RESULTS

A total of 268 cases of autoimmune bullous diseases were registered in the Immunobullous Disease Clinic during the study period, of which 50 (18.7%) were subepidermal. Bullous pemphigoid was the most common subepidermal autoimmune bullous disease observed in 20 (40%) patients, followed by dermatitis herpetiformis in 14 (28%), mucous membrane pemphigoid in 6 (12%), chronic bullous dermatosis of childhood / linear immunoglobulin A bullous dermatosis in 5 (10%), lichen planus pemphigoides in 3 (6%), and pemphigoid gestationis and epidermolysis bullosa acquisita in 1 (2%) case each. The clinico-pathological and treatment details

| SABD | Clinical | Histopathological | DIF |
|------|----------|-------------------|-----|
| BP   | Tense bullae/urticarial lesions predominantly on flexures, ± milia and absence of scarring | Subepidermal bulla with eosinophils ± neutrophils in upper dermis | Linear IgG and/or C3 ± IgM, IgA at BMZ |
| LPP  | Lichenoid papules, tense bullae on both lichenoid papules and clinically uninvolved skin | Subepidermal bulla with eosinophils ± neutrophils, civatte bodies, basal layer degeneration, dense band-like infiltrates, pigment incontinence | Bulla: same as BP Lichenoid papule: IgM in civatte bodies, shaggy fibrinogen at BMZ |
| PG   | Tense bullae/urticarial/erythema multiforme-like lesions on periumbilical area and elsewhere in pregnancy / postpartum | Same as BP | Predominance of C3 over IgG at BMZ |
| MMP  | Oral / ocular / genital erosions associated with scarring ± cutaneous lesions | Same as BP | Same as BP |
| LABD/ | Tense blisters often in a string of pearls arrangement | Same as BP | Linear IgA ± IgG at BMZ |
| CBDC | | | |
| EBA  | Tense bullae predominantly at acral sites associated with milia and scarring | Subepidermal bulla with neutrophils | Same as BP |
| BSLE | Tense bullae on urticarial plaques on sun-exposed and sun-protected sites in established SLE | Subepidermal bulla with neutrophilic infiltrate | IgG, IgA, IgM and C3 (granular, band like) at BMZ |
| DH   | Papulo-vesicles associated with intense itching on elbows, knees, buttocks, scapular region | Subepidermal blister with papillary tip neutrophilic microabscess | Granular IgA at papillary tips |

SABD: Subepidermal autoimmune bullous disease, DIF: Direct immunofluorescence, BMZ: Basement membrane zone, BP: Bullous pemphigoid, LPP: Lichen planus pemphigoides, PG: Pemphigoid gestationis, MMP: Mucous membrane pemphigoid, LABD: Linear IgA bullous dermatosis, CBDC: Chronic bullous dermatosis of childhood, EBA: Epidermolysis bullosa acquisita, BSLE: Bullous systemic lupus erythematosus, DH: Dermatitis herpetiformis, SLE: Systemic lupus erythematosus, IgA: Immunoglobulin A, IgM: Immunoglobulin M, IgG: Immunoglobulin G
of classical and atypical bullous pemphigoid patients [Figures 1-4] are shown in Table 2a. The demographic, clinical and treatment profile of patients with bullous pemphigoid, dermatitis herpetiformis [Figures 5 and 6], mucous membrane pemphigoid [Figure 7], chronic bullous dermatosis of childhood and lichen planus pemphigoides [Figure 8] are summarized in Table 2b. The only woman with linear immunoglobulin A bullous dermatosis was diagnosed clinically as bullous pemphigoid. She also had oral and conjunctival lesions. Her histopathology revealed a subepidermal bulla with an equal proportion of eosinophils and neutrophils. Direct immunofluorescence showed linear deposits of immunoglobulin A (4+) and immunoglobulin G (2+) with the absence of C3 at dermoepidermal junction. Based on these findings, her diagnosis was revised to linear immunoglobulin A bullous dermatosis. She showed good improvement with oral prednisolone and dapsone. The clinicopathological and treatment details of indeterminate cases are shown in Table 3. Figure 9 depicts the clinical presentation in a patient with epidermolysis bullosa acquisita.

**DISCUSSION**

There is a paucity of demographic and clinicopathological studies on different subepidermal autoimmune bullous diseases from India. The present study describes the demographic and clinicopathological features of various subepidermal autoimmune bullous diseases observed in a clinic setting and their comparison with other countries [Table 4]. Bullous pemphigoid was the most common subepidermal autoimmune bullous disease which is in accordance with studies from Kuwait, France, Germany and Singapore.[1-4] However, the proportion of bullous pemphigoid cases was lower compared to that in European countries and Singapore.[2-4] The mean age at diagnosis was 59 years which was similar to that from Singapore (58.4 years) and slightly lower than that reported by Nanda et al. (65.97 years).[1,5] On the other hand, studies from Europe have documented a much higher mean age at diagnosis (73.7 and 82.4 years).[2,3] We observed almost equal gender distribution similar to that reported from Germany and Singapore, while female preponderance was noted in Kuwait (1:6) and France (1:1.5).[3,5] Majority of our patients (65%) had mild disease, unlike moderate to severe disease reported...
in 96% of cases from Kuwait. The incidence of mucosal involvement was 40% in the present study which is almost similar to that described from Kuwait (37%), but significantly higher compared to France and Taiwan (24.6% and 12.8%, respectively).

We had one patient with a provisional diagnosis of anti-p200 pemphigoid that was associated with psoriasis. A confirmed diagnosis was not possible due to lack of indirect immunofluorescence and immunoblotting facilities. Anti-p200 pemphigoid is a rare subepidermal autoimmune bullous disease that may clinically resemble bullous pemphigoid, linear immunoglobulin A bullous dermatosis, dermatitis herpetiformis or pemphigus herpetiformis and histopathologically shows subepidermal bulla with a collection of neutrophils in the papillary dermis. Indirect immunofluorescence on salt-split skin demonstrates autoantibodies binding along the dermal side of the split. Anti-p200 pemphigoid sera react to laminin γ1 on Western blotting in 90% of patients. Psoriasis has been associated in 30% of these cases. Two of our patients had a seizure disorder. An Indian patient having dyshidrosiform bullous pemphigoid associated with parkinsonism has been described. It has been suggested that bullous pemphigoid antigen-1n (neuronal isoform) gets unmasked in the setting of a neurodegenerative disease resulting in antibodies that cross-react with the epithelial form of bullous pemphigoid antigen-1e. Langan et al. reported the development of bullous pemphigoid in 21% of cases with neurological complaints such as stroke, dementia, parkinsonism,

**Table 2a: Clinico-pathological and treatment details of classical and atypical bullous pemphigoid patients**

| BP          | Clinical features                                                             | Provisional diagnosis | H and E          | DIF C3 IgG IgA | Other investigations | Final diagnosis | Treatment                                      |
|-------------|-------------------------------------------------------------------------------|-----------------------|------------------|----------------|---------------------|-----------------|------------------------------------------------|
| Classical   | Tense bullae/urticarial lesions predominantly on flexures, ± milia and absence of scarring | BP                    | Subepidermal bulla with eosinophils ± neutrophils in upper dermis | 100% 85% 50% | Peripheral blood eosinophilia in 1 case | BP              | Oral prednisolone, azathioprine, dapsone, rituximab |
| Atypical case 1 | Disseminated DLE with 4/11 ARA criteria for SLE. Tense bullae, both clear fluid-filled and hemorrhagic, on normal skin as well as on DLE lesions | (a) BSLE (b) BP | Subepidermal bulla with blister cavity containing neutrophils with few eosinophils | 2+ 3+ - | ANA - 3+ (speckled) Anti-ds DNA - negative | BP with disseminated DLE | Oral prednisolone (30 mg/day) and hydroxychloroquine (400 mg/day) |
| Atypical case 2 | Known case of psoriasis. Tense bullae all over the body including oral and genital mucosa for 15 days | (a) BP (b) Anti-p200 pemphigoid | Subepidermal bulla with cavity containing polymorphs admixed with eosinophils | 3+ 3+ - - | Anti-ds DNA - negative | Anti-p200 pemphigoid | Oral prednisolone (30 mg/day) and methotrexate (25 mg/week) |

H and E: Hematoxylin and eosin, DIF: Direct immunofluorescence, ARA: American Rheumatism Association, BP: Bullous pemphigoid, BSLE: Bullous systemic lupus erythematosus, DLE: Discoid lupus erythematosus, DEJ: Dermo-epidermal junction, ANA: Anti-nuclear antibody, ds DNA: Double stranded deoxy-ribonucleic acid.

Figure 5: (a) Grouped excoriated erythematos papules healing with hypopigmentation on the elbows in dermatitis herpetiformis. (b) Similar lesions distributed on the knees.

Figure 6: (a) Subepidermal split with blister cavity rich in neutrophils in dermatitis herpetiformis (H and E, ×100). (b) Direct immunofluorescence showing granular deposits of immunoglobulin A in the dermal papillae.
epilepsy and multiple sclerosis. A single case of malignancy (squamous cell carcinoma of the tongue) associated with bullous pemphigoid was seen in our study. Fernandes et al. from India reported squamous cell carcinoma of the tongue with lymph node metastasis in a patient of bullous pemphigoid whose disease course paralleled that of malignancy. Chang et al. from Taiwan found internal malignancy in 15.1% of their cases of bullous pemphigoid but the association was not statistically significant when compared with age and gender matched controls. Patients refractory to standard therapies should be evaluated for underlying malignancy. In our study, hypertension (80%) and diabetes (35%) were the most commonly encountered systemic diseases, much higher than the rates from Singapore (21.6% and 14% respectively). An age and gender matched control group should be studied to determine any significant association of bullous pemphigoid with these diseases which are otherwise common in the older population. C3 was the most frequent immunoreactant at the dermoepidermal junction followed by immunoglobulin G and immunoglobulin A in our bullous pemphigoid patients. This is in accordance with the study from Singapore which found C3 (87.8%) followed by immunoglobulin G (51.5%) as the most common reactants. In 29.7% of cases, C3 alone was detected in the absence of immunoglobulins and in 8.1%, direct immunofluorescence was negative. Inchara and Rajalakshmi from India reported the sensitivity of direct immunofluorescence to be 82% (23/28) in bullous pemphigoid among a study on 100 patients of immunobullous diseases. Another Indian study evaluated the role of salt-split skin as a substrate for direct and indirect immunofluorescence in 32 histopathologically confirmed cases of bullous pemphigoid.

Table 2b: Demographic, clinical and treatment profile of patients with various subepidermal autoimmune bullous diseases

| SABD  | Total patients, n | Mean age in years (range) | Male: Female | Mean duration (range) | Mucosal involvement (%) | Associated diseases (%) | Treatment |
|-------|-------------------|---------------------------|--------------|-----------------------|-------------------------|-------------------------|-----------|
| BP    | 20                | 59 years (33-80 years)    | 1.2:1        | 2.5 years (1 month-26 years) | 8/20 (40)               | Hypertension: 10 (50)  | Prednisolone, azathioprine, dapsone, rituximab |
| DH    | 14                | 41.5 years (17-75 years)  | 0.8:1        | 4.5 years (6 months-20 years) | Genital (7.1)            | Symptomatic celiac disease 2 (14.3) | Dapsone and gluten free diet |
| MMP   | 6                 | 60 years (48-76 years)    | 1:5          | 3.9 years (1-7.5 years)    | Oral (83.3)              | Hypertension 3 (50) Oral  (66.7) | Prednisolone, cyclophosphamide, dapsone, azathioprine, rituximab |
| CBDC  | 4                 | 4.3 years (1-7 years)     | 3:1          | 5.4 months (1.5-10 months) | Genital and oesophageal (16.7) | None                    | Dapsone, prednisolone |
| LPP   | 3                 | 62.3 years (53-70 years)  | 1:2          | 7.2 years (1.5-16 years)   | Oral (66.7)              | Autoimmune thyroiditis and hypertension 1 (33.3) | Prednisolone, azathioprine |

SABD: Subepidermal autoimmune bullous disease, BP: Bullous pemphigoid, SCC: Squamous cell carcinoma, LPP: Lichen planus pemphigoides, MMP: Mucous membrane pemphigoid, CBDC: Chronic bullous dermatosis of childhood, DH: Dermatitis herpetiformis, DLE: Discoid lupus erythematosus
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They observed equal sensitivity of direct immunofluorescence on the non salt-split skin and salt-split skin though additional immunoreactants were identified in some cases with salt-split skin. On the contrary, salt-split skin was twice as sensitive as non- salt-split skin for indirect immunofluorescence.[13]

The incidence of peripheral blood eosinophilia was low (5%) in our study, in contrast to 22.1% observed in Taiwan.[7]

In the present study, dermatitis herpetiformis was the second most common subepidermal autoimmune bullous disease accounting for 28% of the cases. This was in striking contrast to the studies from Kuwait and Singapore where none of the patients had dermatitis herpetiformis.[1,4] This may possibly be due to differences in the human leukocyte antigen patterns in various ethnic groups. Furthermore, our results cannot be compared with those from Europe as they had excluded dermatitis herpetiformis among subepidermal autoimmune bullous diseases.[2,3] The mean age of our patients was 41.5 years which was slightly younger than that reported in a clinico-immunopathologic study of 264 dermatitis herpetiformis patients registered at Mayo Clinic (1970–1996).[14] We observed slight female dominance, in contrast to male dominance in the Mayo Clinic study.[14] Autoimmune hypothyroidism and symptomatic celiac disease were seen in 14.3% cases each. This was comparable to the series from Mayo Clinic where celiac disease was present in 12.6% and autoimmune systemic diseases in 22.2% patients (thyroid disorders in 11.1% patients).[14]

Mucous membrane pemphigoid accounted for 12% of subepidermal autoimmune bullous diseases. This

Table 3: Clinico-pathological and treatment details of indeterminate cases

| Age/sex | Clinical | Provisional diagnosis | H and E | DIF | Treatment |
|---------|----------|-----------------------|---------|-----|-----------|
| 69/male | Conjunctival redness with symblepharon | MMP | Subepithelial fibrosis, no bulla | Negative | Dapsone |
| 52/female | Conjunctival redness with symblepharon | MMP | Subepithelial fibrocollagenous tissue with mild inflammation, no bulla | Negative | Prednisolone, cyclophosphamide |
| 46/female | Oral erosions | BP MMP Lichen planus | Subepithelial blister with hemorrhage | Negative | Dapsone |

H and E: Hematoxylin and eosin, DIF: Direct immunofluorescence, BP: Bullous pemphigoid, MMP: Mucous membrane pemphigoid

Table 4: Comparison of proportion of subepidermal autoimmune bullous diseases in our study with other studies

| Variable | Current study | Wong and Chua[4] | Nanda et al.[1] | Bernard et al.[2] | Zillikens et al.[3] |
|----------|---------------|-----------------|-----------------|-----------------|-------------------|
| Study period | 2013-14 | 1998-99 | 1991-2002 | 1989-1990 | 1989-94 |
| Total SABDs (n) | 50 | 67 | 68 | 100 | 80 |
| BP (%) | 40 | 88 | 40 | 69 | 76.3 |
| DH (%) | 28 | - | - | Not included | Not included |
| MMP (%) | 12 | - | 1.5 | 12 | 10 |
| CBDC/LABD (%) | 10 | 3 | 13 | 5 | 2.5 |
| LPP (%) | 6 | - | 6 | 5 | - |
| EBA (%) | 2 | 4.5 | 4.5 | 2 | 2.5 |
| PG (%) | 2 | - | 35 | 4 | 6.3 |
| BSLE (%) | - | 3 | - | 2 | - |
| Indeterminate (%) | 3 cases | - | - | 6 | 2.5 |

SABD: Subepidermal autoimmune bullous disease, BP: Bullous pemphigoid, LPP: Lichen planus pemphigoides, PG: Pemphigoid gestationis, MMP: Mucous membrane pemphigoid, LABD: Linear IgA bullous dermatosis, CBDC: Chronic bullous dermatosis of childhood, EBA: Epidermolysis bullosa acquisita, BSLE: Bullous systemic lupus erythematosus, DH: Dermatitis herpetiformis, IgA: Immunoglobulin A

Figure 9: (a) Crusted erosions healing with scarring and milia on the knees in epidermolysis bullosa acquisita. (b) Elbows showing similar morphology of lesions
was comparable to that reported from France and Germany (12% and 10%, respectively) where mucous membrane pemphigoid is the second most frequent subepidermal autoimmune bullous disease after bullous pemphigoid.[2-3] In contrast to this, only 1.5% cases of mucous membrane pemphigoid were reported from Kuwait and none from Singapore.[1,4] In the present study, mean age of the patients was 60 years, a slightly lower figure when compared to that described from Europe (69 and 73 years).[2,3] The female preponderance in our study was similar to that observed in Germany (1:7), although in the French study, males were more frequently affected (1:0.3).[2,3] We found ocular mucosa to be most frequently affected followed by oral, genital and esophageal mucosa. A study from Mayo Clinic on 81 patients of mucous membrane pemphigoid reported oral lesions in 84%, ocular in 76.5%, genital in 29.6% and esophageal in 7.4% patients.[15] In our study, hypertension and diabetes mellitus were present in 50% and 33.3% patients, respectively and malignancy in none. On the other hand, at Mayo Clinic, malignancy was observed in 14.8% cases though the association was not significant. Diabetes and hypertension were observed in 4.9% cases each.[15]

Although lichen planus pemphigoides was earlier thought to be an expression of coexisting lichen planus and bullous pemphigoid, it is now regarded as a distinct entity. Compared to bullous pemphigoid, it affects a younger age group, has a tendency to involve the lower extremities and has a less severe course. In the present study, lichen planus pemphigoides contributed to 6% of the total subepidermal autoimmune bullous diseases. Studies from France, Germany and Singapore did not observe any case of lichen planus pemphigoides.[2-4] Although mostly idiopathic, lichen planus pemphigoides may be associated with drug intake, phototherapy, hepatitis B/C and internal malignancy. One of our patients had autoimmune hypothyroidism. On histopathology, we found civatte bodies in all three patients while Zaraa et al. described civatte bodies in 16.7% and fibrin in 11.5% patients.[16] The other subepidermal autoimmune bullous diseases (pemphigoid gestationis, epidermolysis bullosa acquisita and bullous systemic lupus erythematosus) were uncommon as observed in similar studies from elsewhere. The limitations of our study were that we could not perform immunofluorescence on salt-split skin and immunoblot analysis which would have helped in further characterization of cases that could not be diagnosed based on histopathology and direct immunofluorescence alone and would have prevented any possible misclassification of the remaining cases.

CONCLUSION

Bullous pemphigoid is the most common subepidermal autoimmune bullous disease in India as in Western Europe though the proportion, at least in our cohort of patients, seems to be lower, with an earlier age of onset. The second most frequently encountered subepidermal autoimmune bullous disease is dermatitis herpetiformis, in contrast to the Far East and European countries. The other subepidermal autoimmune bullous diseases such as mucous membrane pemphigoid, linear immunoglobulin A bullous dermatosis, lichen planus pemphigoides, pemphigoid gestationis, epidermolysis bullosa acquisita and bullous systemic lupus erythematosus are relatively less common in our patients. Larger studies on the epidemiology and clinico-immunological profile of individual subepidermal autoimmune bullous diseases in Indian patients will help in characterizing them better.

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Conflicts of interest
There are no conflicts of interest.

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