



## Pemphigus – A Clinicopathological Study

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### Abstract

**Introduction:** Pemphigus refers to a group of chronic cutaneous autoimmune blistering diseases caused by antidesmosomal antibodies leading to acantholysis with consequent blisters.

**Aims:** To study the clinical, cytological, histopathological and immunofluorescence findings in pemphigus.

**Methods:** Thirty patients with pemphigus were studied. Tzanck smear could be done in 25 patients. Histopathology examination was carried out in 29 cases. Direct immunofluorescence was done in 29 patients. All the data were analysed using the computer software, statistical package for social science (SPSS) version 10.

**Results:** Of the 30 pemphigus patients studied, majority of males (53.3%) had onset in the age group 30-44 years and 53.3% of females had onset at a slightly higher age group. In 5 cases, onset of disease was noted in the age group >60 years. Two cases gave history of vesiculobullous disease in their siblings. Diabetes was present in 23.3% cases, thyroid disorders in 16.7% and hypertension in 10%. 40% showed *Staphylococcus aureus* growth, 13.7% with Methicillin resistant *staphylococcus aureus* and 6.3% had streptococcal growth. Tzanck smear was positive in 88%. By histopathological examination all the patients could be diagnosed as either pemphigus vulgaris or pemphigus foliaceus. In DIF study, 96.6% showed intercellular IgG and 86.2% cases showed C3.

**Conclusion:** All clinically diagnosed cases could be confirmed either by histopathology or DIF as pemphigus. Identification of type of pemphigus was possible by histopathology in 100% cases where it was done.

**Keywords:** pemphigus, Tzanck, Direct immunofluorescence, histopathology.

### Introduction

The term pemphigus represents a group of autoimmune blistering diseases of skin and mucous membranes, characterized histologically by intraepidermal blisters due to acantholysis and immunopathologically by in vivo bound and circulating IgG directed against desmogleins, which are adhesion molecules functioning in desmosomes of the keratinocytes. The peak incidence of pemphigus vulgaris occurs between

the fourth and sixth decades of life. Genetic susceptibility to pemphigus is apparent from factors such as, its increased prevalence among Jewish or Mediterranean ancestry, presence of familial cases and increased occurrence of other autoimmune diseases in the first-degree relatives of pemphigus patients .

Autoimmune disorders reported to be associated with pemphigus include thymoma, myasthenia gravis, autoimmune thyroid disease, diabetes,

rheumatoid arthritis, lupus erythematosus and pernicious anaemia. The exact pathogenesis of Multiple autoimmune syndrome (MAS) is not known. It has been stated that there is an increased tendency for development of a new autoimmune disorder in individuals with previous history of an autoimmune disease.<sup>1</sup> An explanation given for MAS is the sharing of common genetic elements related to the different diseases. Other possible reasons suggested are the presence of common autoantibodies directed against myoepithelial cells and also diminished specific suppressor T cell function.

Investigations for diagnosis include Tzanck smear, histopathology examination and immunofluorescence tests. **Tzanck smear** is a rapid and inexpensive test. Skin biopsy is advised from a fresh bulla. If no recent blister is present, an old one may be moved by vertical pressure with a finger into the neighbouring skin to create a new area of cleavage. Direct immunofluorescence (**DIF**) supplements clinical findings and histopathology in diagnosis and permit early diagnosis. Fluorescein isothiocyanate and tetramethylrhodamine isothiocyanate are the routinely used fluorochromes. Pemphigus vulgaris and pemphigus foliaceus display similar findings. So they cannot be differentiated by DIF always. They show intercellular deposition of IgG and C3 giving a "fish-net" pattern.

A less invasive and relatively simple alternative to DIF on a skin biopsy, is DIF on the outer root sheath (ORS) of plucked hairs because hair follicle with its ORS has greater density of pemphigus antigens. DIF of hair can be used as an additional procedure for assessment of immunological remission in pemphigus vulgaris.<sup>2</sup> There are some anatomic oral regions in which the DIF sensitivity is greater which may allow the clinician to choose accessible regions for biopsy. These sites include floor of mouth, hard palate, superior labial mucosa, and ventral side of the tongue.<sup>3</sup>

Much changes have occurred in the immunological understanding of the disease in the

recent years. Apart from skin and mucosal biopsy, cytological study, direct and indirect immunofluorescence and ELISA for direct measurement of antibodies to desmogleins in serum are available now. So our objective is to study the clinical profile, cytological, histopathological and immunological findings of pemphigus and its variants.

### Materials & Methods

During the one and half year period, 30 patients were studied. Detailed history was taken and clinical examination was done after getting consent. Diagnosis was confirmed by cytology, histopathology or by direct immunofluorescence studies. Tzanck smear could be done in 25 patients and 2 had only oral erosions. Histopathology examination (HPE) was carried out in 29 cases. Direct immunofluorescence (DIF) was done in 29 patients. As facilities are not available in our hospital, specimens were posted in Michels medium to a distant hospital.

Apart from the routine blood and biochemical parameters, Chest X-ray, Ultrasound scan abdomen and Thyroid function tests were done to rule out systemic disorders. Pus culture and sensitivity was also done in 15 patients. Herpes simplex virus type 1 antibody titer was done in 2 patients who had only oral lesions.

All the data were entered in the proforma and were analysed using the computer software, statistical package for social science (SPSS) version 10. The data was given in frequency and percentage. A two-tailed distribution was assumed for all statistical evaluations and probability value <0.05 was considered significant.

### Results

Among the 30 pemphigus patients studied, 20 patients (66.67%) had the age of onset between 30-59 years. Majority of males (53.3%) had onset of the disease in the age group 30-44 years and majority (53.3%) of females had onset at a slightly higher age group of 45-59 years. In 5 cases

(16.65%), onset of the disease was noted in the older age group (>60 years). Majority were housewives (33.3%), next group being manual labourers (20%) as shown in Table 1.

One patient (3.3%) had history of papillary carcinoma thyroid which was treated 16 years back. Two index cases gave history of vesiculobullous disease in their siblings. Family history of diabetes mellitus was present in 10 cases, malignancy in 3 and thyroid disease in 2 patients. Upper trunk (89.3%) was the commonly involved site. Frequency of involvement of various sites in oral mucosa is shown in Table 2.

Diabetes was present in 23.3% cases, atopy in 13.3%, hypertension in 10%, hypothyroidism in 13.3% cases and 3.3% showed hyperthyroidism. Malignancy was not detected in any of the patients. Among 15 patients in whom pus culture and sensitivity was done, 40% showed Staphylococcus aureus growth, 13.7% with Methicillin resistant staphylococcus aureus (MRSA) and 6.3% had streptococcal growth.

Of the 25 patients where Tzanck smear could be done, 88% were positive for acantholytic cells. By histopathological examination (HPE) all the 29 patients could be diagnosed as either pemphigus vulgaris or pemphigus foliaceus by the characteristic suprabasal blister and subcorneal blister respectively. The case in which histopathology could not be done was diagnosed as pemphigus vulgaris clinically and cytologically. Other findings noted in HPE include acantholytic cells (79.3%), epidermal villi (34.5%), inflammatory cells (27.6%), 'row of tombstone' appearance (13.8%), necrotic cells (10.3%) and spongiosis (3.4%).

DIF study was done in 29 patients (Table 3). In one patient report came as not suggestive of pemphigus, which may be due to a technical error. In this patient histopathology report was consistent with pemphigus vulgaris and Tzanck smear demonstrated acantholytic cells. In another case, both IgG and IgM were found at basement membrane zone, suggesting paraneoplastic pemphigus. With the available investigations, no

malignancy was found. So it was diagnosed as pemphigus vulgaris.

Diagnosis was confirmed either by cytology, histopathology, DIF or by all these. Identification of type of pemphigus was possible by histopathology in 100% cases where histopathology was done. This was possible mainly by observing the level of blister. All cases clinically diagnosed as pemphigus were confirmed either by histopathology or DIF as pemphigus.

**Table 1** Occupation

Occupation	Count	Percent
Manual labourer	6	20.0
Skilled worker	5	16.7
White collar job	4	13.4
Business	1	3.3
House-wife	10	<b>33.3</b>
Student	4	13.3

**Table 2** - Oral sites

Oral sites	Count	Percent
Buccal	21	<b>80.8</b>
Tongue	6	23.1
Lips	17	65.4
Palate	7	26.9
Gingiva	8	30.8

**Table 3** Direct immunofluorescence

Direct immunofluorescence	Count	Percent
IgG -intercellular	28	96.6
IgG - BMZ	1	3.3
C3	25	86.2
IgM	1	3.3
IgA	0	0.0
Fibrinogen	0	0.0

## Discussion

Out of 30 pemphigus cases, in 53.3% of males, onset of the disease was found in the age group 30-44 years and females had a slightly older age of onset of 45-59 years. This was similar to the findings of Naseer SY et al, where male patients presented with pemphigus vulgaris before 40 years of age whereas females commonly presented between the ages of 40-49 years.<sup>4</sup> Onset of the disease was noted in the older age group (>60 years) in 16.65% patients in our study. More number of patients (29.26%) presented after 60 years of age, according to Chowdhury J et al.<sup>5</sup>

In our study, majority of the cases were homemakers (33.3%) followed by labourers (20%) and skilled workers (16.7%). Asia AJ et al reported much higher rate (59% Vs33.3%) of pemphigus among housewives but conforming to our study among workers (17.9 % Vs 16.7%).<sup>6</sup> Family history of pemphigus was present in 6.7 % - a sibling was affected in both in the present study. Positive family history was found in 1.4% cases in a 10 year retrospective study done in Iran by Javidi Z et al which is not comparable to the present study.<sup>7</sup>

Among our study population, past history of thyroid carcinoma was present in one patient (3.3 %). But in the population based study done in Israel there was a greater prevalence of esophageal cancers (0.4% vs 0.1%) and laryngeal cancers among pemphigus cases (0.6% vs 0.3 %).<sup>8</sup> In the study group, buccal mucosa was the most commonly affected site (80.8%) , followed by lips (65.4%), gingiva (30.8%),palate (26.9%). Tongue was the least common site involved (23.1%). Observations by Shamim T et al was not conforming to the present study where they got buccal mucosa (90.14%) followed by palate and lips (50.70%), tongue (28.17%), floor of mouth (23.94%) and gingiva (21.12%).<sup>9</sup>

In the present study, diabetes was present in 23.3%, thyroid diseases in 16.7%, atopy in 13.3% and hypertension in 10%cases. Frequency of comorbidities according to PN Mini et al was not similar to our study which included diabetes mellitus (51 %), hypertension (30.2 %), thyroid disease (11.6 %), and bronchial asthma (9.3%).<sup>10</sup> Persons with an autoimmune disease should be monitored for development of related diseases.<sup>11</sup>

Variable degree of bacterial infection occurs in some cases. Out of 15 patients, 40% showed Staphylococcus aureus growth, 13.7% with Methicillin resistant staphylococcus aureus (MRSA) and 6.3% had streptococcal growth. The present study was not in accordance with the results of Esmaili N et al where out of 155 cases, 16 had skin infections. Among them, majority had staphylococcal infection (93.7 %).<sup>12</sup>

Tzanck smear was positive in 88% of our patients. The study by Basu K et al <sup>13</sup> (88.24 %) was consistent with ours while the findings of Ljubojević S et al<sup>14</sup> (72 %) was not conforming. Histopathology findings noted in our 29 patients include suprabasal blister (89.6%),subcorneal blister (10.3%), acantholytic cells(79.3%), epidermal villi (34.5%), inflammatory cells (27.6%), 'row of tombstone appearance (13.8%) and spongiosis in 3.3 %. In few other studies, number of cases showing similar histopathological findings was not consistent with our study. Mahajan et al found presence of suprabasilar split, acantholytic cells and dermal infiltrate comprised of neutrophils and lymphocytes in 100% patients, spongiosis in 88.6% and tombstone appearance in 62.86%.<sup>15</sup> According to Mini PN et al, the blisters demonstrated acantholytic cells in 76.74%, neutrophils in 23.26%, eosinophils in 13.95%, and lymphocytes in 4.65%, also row of tombstone appearance in 25% specimens and villi formation was seen in 19.44% patients.<sup>10</sup>

In DIF test, 96.6% showed intercellular IgG and 86.2% cases showed C3 deposition among our cases. In contrast, in the study done by Mimouni *et al*, DIF test showed deposition of IgG and C3 in the intercellular spaces in 71.4% and variable immunostaining at the basement membrane zone in 42.8%.<sup>16</sup>And DIF findings demonstrated by Arbache ST et al were IgG (ICS) and C3 for PF (94% and 73% respectively ) and in pemphigus vulgaris (91.5%-79.5%).<sup>17</sup>

By histopathology examination, identification of type of pemphigus was possible in 100% cases in the present study. All cases clinically diagnosed as pemphigus were confirmed either by histology or DIF as pemphigus. Similarly, Arundhathi S et al concluded that histopathology remains the cornerstone in differentiating PV from PF and DIF is helpful in scenarios where clinical and/ or histopathological features are inconclusive.<sup>18</sup>But from the Eastern India study, it was seen that almost 85.36% cases of pemphigus were diagnosed clinicopathologically. The six cases which could not be confirmed on

clinicopathological basis were correctly diagnosed by DIF.<sup>5</sup> DIF has got some prognostic value also. Negative findings during remission may be considered as a good prognostic factor.<sup>19</sup>

### Summary & Conclusion

Screening of patients and family members may be done for occurrence of autoimmune diseases to decrease morbidity and mortality associated with MAS. It is shown that, histopathological examination is more helpful in classification of pemphigus. Immunofluorescence study can be a useful tool for confirmation of diagnosis when histopathology is inconclusive.

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