ORIGINAL RESEARCH

EVALUATION OF DSG1 AND DSG3 MOLECULES IN PEMPHIGUS CASES

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ABSTRACT:
Introduction: Autoimmune blistering diseases are a rare group of mucocutaneous disorders that can result in irreversible sequelae and death if accurate diagnosis and treatment are not rendered promptly.1, 2 Two of the most common diseases in this group are pemphigus vulgaris and bullous pemphigoid, which are disorders characterized by the production of autoantibodies that target structural proteins important to the maintenance of intercellular and cell-to-basement membrane adhesion. **Material & Methods:** Sixty patients with pemphigus (40 with PV and 20 with PF, who were diagnosed based on clinical examination, histopathology and direct immunofluorescence) and three patients with extensive burns which were considered as controls were studied. **Results:** For Dsg1 ELISA, the mean was 0.143 and for Dsg3 it was 0.102. This resulted in a cut off value of 0.406 for Dsg1 ELISA and 0.212 for Dsg3 ELISA. Dsg1 and Dsg3 antibodies were found to be present in all the patients of PV and PF. **Conclusion:** A detailed prospective study for evaluating the ELISA levels of a given patient for prolonged periods would be helpful in actual correlation with the disease severity and changes that may occur in the spectrum of the disease.
Keywords: Pemphigus, DSG1, DSG3, ELISA

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INTRODUCTION

Autoimmune blistering diseases are a rare group of mucocutaneous disorders that can result in irreversible sequelae and death if accurate diagnosis and treatment are not rendered promptly.[1]Two of the most common diseases in this group are pemphigus vulgaris and bullous pemphigoid, which are disorders characterized by the production of autoantibodies that target structural proteins important to the maintenance of intercellular and cell-to-basement membrane adhesion.[2]Diagnosis of these disorders requires the integration of clinical findings, histopathologic characteristics, immunofluorescent analysis, and further immunologic laboratory testing (eg enzyme-linked immunosorbent assay) if necessary.[3]

Pemphigus vulgaris is a rare autoimmune disease (up to 3.2 cases per 100,000 population) that causes severe blistering of the skin and of the mucous membranes lining the mouth, nose, throat and genitals.[4] Blisters are sacs with fluid that develop on the upper layer of the skin so their roofs are very thin and fragile, and break easily to leave raw areas (erosions) that can be extensive and painful. Pemphigus does not go away by itself, and always needs treatment by a Dermatologist.

Pemphigus has several subtypes, of which three have been associated with oral mucosal involvement; pemphigus vulgaris (PV), pemphigus foliaceus (PF), and paraneoplastic pemphigus.[5] The first two subtypes are differing with respect to the localization of intraepithelial blisters. In PV, the blisters are located suprabasally, while in PF they are more superficially located. Paraneoplastic pemphigus, although uncommon, is associated with internal malignant neoplasia.[6]

Pemphigus is one of the best-characterized tissue-specific autoimmune diseases. The desmoglein proteins desmoglein-3 (Dsg3) and desmoglein-1 (Dsg1) have been identified as the antigens in pemphigus vulgaris (PV) and pemphigus foliaceus (PF) respectively.[7] The production of recombinant Dsg1 and Dsg3 molecules has provided the opportunity to determine levels of antibodies to them (anti-Dsg1 IgG and anti-Dsg3 IgG respectively) and to see if they correlate with disease severity. However, there are conflicting data in this regard in many studies using immunofluorescence titers.[7]

ELISA is a simple and effective tool for the quantitative analysis of antibody levels. Harman et al. used ELISA to measure serial Dsg1 and Dsg3 antibody levels and correlate them with the severity of oral and skin ulceration in PV and PF.[7] As there are hardly any similar studies from India, we conducted this cross-sectional study to correlate the severity of oral and/or cutaneous involvement in patients with PV and PF with ELISA values for antibodies to Dsg1 and Dsg3 independently.

METHODS

Sixty patients with pemphigus (40 with PV and 20 with PF, who were diagnosed based on clinical examination, histopathology and direct immunofluorescence) and three patients with extensive burns which were considered as controls were studied. Patients with burns were choosen as controls because pemphigus-like circulating intercellular antibodies have been found in such patients. ELISA was used to confirm the presence of Dsg1 and Dsg3 autoantibodies in PF and PV patients and an attempt was made to correlate their levels with cutaneous or mucosal involvement and disease activity. Ethical clearance was taken from the institute committee. The blood samples were taken from patients with consent form who were untreated and with active disease or from those with resolving disease on treatment of varying durations. No serial samples were taken from any patient.

A simple arbitrary scoring system was used to grade the severity of skin or mucosal involvement at the time of sampling as follows: Oral score 0 = No mucosal involvement. 1 = Minimal disease (only buccal mucosal, labiogingival, lingual, palatal or pharyngeal involvement). 2 = Moderate disease (buccal and labiogingival, lingual, palatal or pharyngeal involvement). 3 = Severe disease (extensive oral erosions, i.e., >3 mucosal sites affected) Skin score 0 = Quiescent disease. 1 = Minimal disease (≤10% body surface area involvement (BSA) involved). 2 = Moderate disease (11-30% BSA involved). 3 = Severe disease: (>30% BSA involved). Antibodies to Dsg1 and Dsg3 were detected in the sera of patients by the micro ELISA technique using kits from Medical and Biological Laboratories Co. Ltd., Japan, as per the manufacturer's instructions. ODs were adjusted relative to the reference sera supplied with the kits. Sixty serum samples were analyzed. The serum samples were randomly taken and subsequently stored at -20o C before processing them for ELISA. The unpaired t test was used to compare Dsg1 and Dsg3 levels in relation to severity of either skin or mucosal lesions.

RESULTS

A cut off value for each ELISA was established from the mean ± 2SD of the three patients with extensive burns. Although the cut off values given in the kit were taken as standard, to increase the sensitivity, we wanted to generate a cut off value from the local population as the kit was manufactured elsewhere. For Dsg1 ELISA, the mean was 0.143 and for Dsg3 it was 0.102. This resulted in a cut off value of 0.406 for Dsg1 ELISA and 0.212 for Dsg3 ELISA. Dsg1 and Dsg3 antibodies were found to be present in all the patients of PV and PF.

For Dsg1 ELISA, 16 of 20 PF patients and 28 of 40 PV patients were above the cut off values. For Dsg3 ELISA, 30 of 40 PV patients and 10 of 20 PF patients exceeded the cut off value.

Table 1: Disease and Demographic parameters

	Pemphigus Vulgaris (n=40)	Pemphigus Foliaceous (n=20)
Males	**22**	**12**
Females	**18**	**8**
Age	**15-60 years**	**20-55 years**
Untreated disease	**24**	**16**
Inactive disease	**16**	**4**

Table 2: Oral disease severity and ELISA levels for antibodies to Dsg1 and Dsg3

Oral mucosal involvement	PV	PF	Mean Dsg1 antibody levels	Mean Dsg3 antibody levels
None	0	20	1.07	0.54
Mild	10	0	0.50	1.10
Moderate	14	0	0.80	2.70
Severe	16	0	1.40	2.80

Table 3: Extent of skin involvement and ELISA levels for antibodies to Dsg1 and Dsg3

Oral mucosal involvement	PV	PF	Mean Dsg1 antibody levels	Mean Dsg3 antibody levels
Mild	8	8	0.40	1.10
Moderate	18	10	1.19	1.90
Severe	14	2	1.80	2.90

We attempted to correlate the extent of skin and mucosal involvement with the independent values of Dsg1 and Dsg3 ELISA. Although a wide range of values was obtained, the general trend was that Dsg3 levels were higher in patients with extensive oral mucosal involvement, i.e., patients with moderate and severe mucosal involvement had significant higher Dsg3 levels than those with mild or no mucosal involvement ($p < 0.1$) [Table 2].

Analysis showed a direct relationship between the severity of skin involvement and the levels of the Dsg1 antibodies, i.e., patients with moderate and severe disease activity had significant higher levels of Dsg1 antibodies when compared to those with minimal disease activity ($p < 0.1$), although patients with extensive disease had slightly more elevated levels of antibodies to Dsg3. However, the presence of severe mucosal involvement correlated well with increased Dsg3 antibody values in PV patients ($p<0.05$). All twenty of our patients with PF (none with mucosal involvement) had detectable levels of Dsg3 antibodies. (Table 3)

DISCUSSION:

The bullous diseases have a history as old as that of medicine. In the early 1950's, Lever was able to differentiate most of these by using histological criteria.[8] The term pemphigus refers to a group of autoimmune blistering diseases of skin and mucous membranes which are characterized histologically by intraepidermal blisters due to acantholysis. Acantholysis is the characteristic feature of the bullae of pemphigus and is defined immunopathologically by the finding in vivo, of bound and circulating IgG directed against the cell surface of keratinocytes.[9]

Pemphigus is a group of chronic inflammatory autoimmune bullous diseases. Although rare, they are potentially life-threatening diseases that are associated with high morbidity and mortality, if not properly treated.[10] The disease is associated with immunoglobulin (Ig) G and complement factor (C) antibodies against intercellular adhesion structural components in the epithelium. The immune reaction eventually breaks down the adhesion components and leads to epithelial cell detachment, which is clinically seen as intraepithelial blisters, erosions or ulcers in the skin and mucous membranes.[11] The underlying cause and activating mechanism that initiates the immune response is unidentified. However, both genetic and environmental factors have been postulated to play a role in the pathogenesis of pemphigus. In this context, social habits like use of traditional cosmetics and smoking have been implicated.

The autoimmune target of pemphigus is desmoglein, a cadherin type of cell-to-cell adhesion molecule found in the desmosomes. There are three isotypes of desmoglein: Dsg1, Dsg2 and Dsg3. Dsg1 and Dsg3 are usually restricted to the stratified squamous epithelia, where blister formation is located in pemphigus. Patients with pemphigus have anti-Dsg1 and/or anti-Dsg3 IgG autoantibodies. IgG1 and IgG4 are the most common subclasses of antibodies in patients with active disease, but IgG4 is more pathogenic and indicates the activity of the disease. Even though PV and PF are distinct diseases, there are several case reports that suggest that a shift between PF and PV may occur. In some of these

reports, immunoblotting studies suggested that the antigen has changed in accordance with the change in clinical picture.[12]

Correlation of the severity of disease with the level of antibodies detected in patients with PV resulted in two interesting observations: Nine out of the 20 patients with PV having quiescent and resolving disease still had high ELISA values for Dsg3 antibodies, although they had been free from active disease for approximately 6 months or more. The reasons for this could be, firstly, the polyclonal nature of the pemphigus autoantibody; therefore, the IgG subclass may be an important determinant. Moreover, up to 70% of the first degree relatives of patients with pemphigus have detectable antibodies that are not of the IgG4 subtype.

In a few cases of PV with active disease, low ELISA values for Dsg1 and Dsg3 were found; it is possible that the sera contained pathogenic antibodies to nondesmoglein molecules or to intracellular domain of Dsg1/Dsg3, which could have been undetectable by the ELISA used to detect specific antibodies to only Dsg 1 and Dsg 3.[12] Our findings suggest that at any given period, the level of the antibodies does correlate with disease severity. However, this may not be true for a small proportion of cases because of the reasons explained above. A detailed prospective study for evaluating the ELISA levels of a given patient for prolonged periods would be helpful in actual correlation with the disease severity and changes that may occur in the spectrum of the disease.

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