Original Article

Immunofluorescence Pattern of Autoimmune Bullous Diseases in Iranian Patients

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ABSTRACT

Background & Objective: **Autoimmune bullous diseases are associated with autoimmunity against structural components in the skin and mucous membranes. Autoantibodies are against the intercellular junctions in pemphigus disease and hemidesmosomal unchoring complex in pemphigiod diseases and epidermolysis bullosa aquisita. The tissue-bound and circulating serum autoantibodies can be detected with direct immunofluorescence (DIF) and indirect Immunofluorescence (IIF) tests. The aim of this study was to pinpoint the immunofluorescence pattern of Iranian patients with autoimmune bullous diseases.**

Methods: **In a prospective case series study, sixteen patients with autoimmune disease enrolled in the study for two years. Perilesional skins and sera from the patients were used in DIF and IIF for detection of immunofluorescence pattern.**

Results: **Out of 16 cases, 9 cases had pemphigus and 7 cases had bullous pemphigoid. All cases of pemphigus had positive DIF in intercellular region with lacelike pattern; IgG was detected in all cases, IgA in 1(11.1%) case and C_3 in 3 (33.3%) cases. One (11.1%) case of pemphigus had positive IIF in intercellular region with lacelike pattern; circulating autoantibodies were IgG and IgA. All cases of bullous pemphigiod had positive DIF in dermal-epidermal Junction with linear pattern. IgG was detected in all cases, IgM in one (14.3%) case, and C3 in six (85.7%) cases. One (14.3%) case of bullous pemphoid had positive IIF in dermal-epidermal Junction with linear pattern; circulating autoantibody was IgG.**

Conclusion: **Immunofluorescence tests are sensitive diagnostic methods for autoimmune bullous diseases. IIF positive cases in our study were lower compared to the previous reports.**

Keywoeds: **Immunofluorescence, Bullous Skin Diseases, Iran**

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Introduction

Autoimmune bullous diseases are associated with autoimmunity against skin structure and mucosal membrane" (1). These structures are responsible for cell to cell and cell to matrix junction. The main characteristic of the pemphigus disease is the presence of autoantibody against cell-to-cell junctions and interaepithelial bulaes. In pemphigiod diseases (bullous pemphigd, Pemphigoid gestations, Linear IgG disease and cicatricial pemphigoid) and Aquiered Epidermolysis Bullosa subepidermal bulaes are associated with auto antibodies against hemidesmosome cell adhesion proteins. The detection of autoantibodies in the tissue and serum is the undispensable part of the diagnosis of autoimmune bullous disease. For that reason, several immunofluorescence assays has been established (1, 2). There are usually two immunofluorescence methods available: direct immunofluorescence (DIF) which usually use for the assessment of immunoreactants in skin or mucosal and indirect immunofluorescence methods (IIF) which is able to detect the antibodies available in serum of the patient using reactions to esophagus of monkey, guinea pig ,rabbit or fresh skin samples (3,4). Currently DIF and IIF are widely being employed for the diagnosis of antibody associated primary bullous diseases (3).

This study was performed to use these methods in an Iranian population in a western province.

Materials and Methods

In a two-year descriptive prospective study (2008-2010), 16 patients with the diagnosis of autoimmune bullous diseases confirmed by clinical and hitopathologic examination using (H&E) stain referred to Farshchian Hospital, Hamedan, Iran enrolled in the study. Blood sample and two skin biopsies (one from the bullous lesion and the other one from adjacent area were taken from each patient. The research protocol was approved by Hamedan University of Medical Sciences Ethics Committee. Following case selection, the study was explained to patients and written consent was obtained according to Ethics Committee permission.

The first sample was fixed in formalin 10% and inspected by light microscopy after H&E staining. Frozen sections were made from the second biopsies in order to use in DIF study. The polyclonal antibody was purchased from Cedarlan (Cedarlan, Canada). The following dilution were used in the experiment: Ig G:1/80, IgM 1/20, IgA 1/40, IgA+M+G 1/40 and C_3 1/40. The sections were incubated in high humidity for 30 min. Following the incubation the sections were washed 3 times with phosphate buffer (PH=7.4) and evaluated with fluorescence microscopic assay (5). Serum samples were used for IIF assay and human normal skin served as the substrate, without fixation-frozen sections with 6μm has made from intact skin. The sections were incubated with different concentrations of diluted and non-diluted from ½ to 1/16 in high humidity for 30 min. Following washing with PBS 3 times, the sections were incubated for 30 min with polyclonal antibodies against IgM, IgG, Ig A and total Ig A+G+M. The sections were inspected with fluorescence microscopic after adding glycerine 50% (6) .

Results

Out of 16 patients, nine (56.2%) had pemphigus and seven (43.8%) were with the diagnosis of bollous pemphigoid disease. The mean age of the patients with pemphigus was 44.2 yr (range: 21-78yr). While the patients with bollous pemphigoid had the mean age of 70.4 yr (range 50-88 yr). Seven out of 9 patients (77.8%) with pemphigus and 6 out of 7 patients (85.7%) with bollous pemphigoid were female. DIF with the lacelike pattern and between the keratinocytes was positive in all patients with pemphigus. IgG, IgA and C_3 deposited was seen in nine (all), 1, and 3 patients respectively. The demographic characteristics of the patients with pemphigus and

the results of DIF test has been shown in table 1. Positive IIF test with lacelike pattern and among the keratinocytes was observed in one patient with pemphigus diagnosis. The autoantibodies in that patient were IgA and IgG types. In all bollous pemphigoid patients DIF with the linear pattern was positive in dermal-epidermal junction. IgG, IgM and C_3 deposited were observed in seven (all), one, and six patients respectively The bollous pemphigoid patient's characteristics and DIF test results has been shown in Table 2. IIF with linear pattern was positive in one of the patients with the detection of IgG.

Table 1- Patients with pemphigus: Characteristics and direct immunofluorescence test findings

Patient code	Age	Gender	IgG	IgM	IgA	Total IgG+M+A	C3
1	61	Female	+	-	-	+	+
2	50	Male	+	-	-	+	+
3	43	Female	+	-	+	+	+
4	78	Female	+	-	-	+	-
5	45	Male	+	-	-	+	-
6	40	Female	+	-	-	+	-
7	32	Female	+	-	-	+	-
8	21	Female	+	-	-	+	-
9	28	Female	+	-	-	+	-

Table 2- Patients with bollous pemphigoid: Characteristics and direct immunofluorescence test findings

Patient code	Age	Gender	IgG	IgM	IgA	Total IgG+M+A	C3
1	88	Female	+	-	-	+	-
2	82	Female	+	-	-	+	+
3	70	Male	+	-	-	+	+
4	57	Female	+	+	-	+	+
5	50	Female	+	-	-	+	+
6	74	Female	+	-	-	+	+
7	72	Female	+	-	-	+	+

Discussion

In this study, 16 patients (9 patients with pemphigus and 7 patients with bollous pemphigoid) enrolled in the study. None of the any other types of autoimmune bullous diseases has been detected during the study period. In similar studies on autoimmune bullous diseases, the most common diseases were pemphigus and bollous pemphigoid (2, 3, 6).

The mean age of the patients with pemphigus in our study was 44.2 yr, which is within the same range reported previously (40-60 years). In contrast to the same previous studies in which both sexes had equal prevalence of the disease, females had higher frequency of the disease compared to men (4). In all patients suffered from pemphigus, DIF with the lacelike pattern and around the keratinocytes was positive like the same pattern observed earlier (2, 6, 7). Although Tan *et al.* have reported DIF deposited in all patients with pemphigus (8), Hong *et al.* have detected DIF deposited in 95% of patients with pemphigud diagnosis and 100% of the activated forms of the disease (3). The results of a meta-analysis study show that IgG deposited can be found in almost all of the patients with

pemphigus, C3 in 50-100 % of patients and IgA or Ig M in 30-50% of the patients (4).

Among our patient with the diagnosis of pemphigus, IIF was positive with the lacelike pattern and around the keratinocytes the detected antibodies were IgA and IgG like the same pattern in previous studies (1, 2, 4). Ahmed *et al.* has reported circulating antibodies in 90% and Hong *et al.* in 80%-90% of the patients (2, 3) which is higher compared to our study. The possible reason for that could be the fact that we have used fresh normal human skin samples instead of monkey oesophagus as the substrate in our study. Like some previous studies the mean age of the patients with bollous pemphigoid was relatively high (70.4 yr) (1,3,4). Although no differences has been reported with respect to the gender of the patients with bollous pemphigoid (1, 3, 4) most of the cases were female in our study. Our results are in good agreement with previous reports in terms of positive DIF test with the linear pattern in dermal-epidermal junction (6, 7, 9). In the present study IgG deposited was observed in all patients with bollous pemphigoid ,C_3 in 85.7% and IgM in 14.3% of the patients. In a meta-analysis study, C_3 deposited has been seen in all patients, Ig G in 65-95% and IgA and IgM in 25% of the cases (3). In another study, the C_3 and IgG or C_3 deposited was dominant (4). C_3 is reported deposited in 85.4% and IgG in 52.7% of the patients with bollous pemphigoid. Similar to other reports, one out of our all patients with bollous pemphigoid had positive linear pattern in dermal-epidermal junction with IgG circulating antibody (2, 3, 10). The circulating antibodies were observed in all patients with bollous pemphigoid (11), 70-80% (2, 3) and 50% of the patients (4). IIF circulating antibodies is seen in 30.9% of the patients (8). The circulating antibodies was IgG in some studies (2,3,8) while in a study not only all the patients had circulating IgG but also circulating IgA was detected in 35% of them (10). Taken together the IIF positive cases in our study is much lower compared to the other reports which using only one substrate (fresh human normal skin) can be one of the possible reasons. IIF using the monkey oesophagus as the substrate has the highest sensitivity in the diagnosis of pemphigus disease. As for the diagnosis of bollous pemphigoid IIF using salt splite skin is a very sensitive test.

Conclusion

Further studies including more patients and recruiting other substrate for IIF such as monkey or guinea pig oesophagus would vividly pinpoint the efficacy of immunofluorescence studies for the diagnosis of autoimmune bullous diseases.

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