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The Role of Anti-skin Antibodies Immunoglobulin G in the Diagnosis of Autoimmune Bullous Diseases

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ABSTRACT

Background: Autoimmune bullous diseases (AIBDs) represent a group of acquired heterogenous organ-specific disorders in which pathogenic autoantibodies, commonly of IgG and/or IgA classes, target adhesion molecules of the skin and mucous membranes. These disorders are caused by the interaction between autoantibodies and structures essential for the integrity of the skin, either on keratinocytes cell surface or in the dermo-epidermal junction, leading to cleavage of the skin at different levels. Consequently, according to the level of cleavage, AIBDs are classified into intraepidermal and subepidermal blistering diseases. Diagnosis of AIBDs depends on the clinical, histological and immunological characteristics of each subtype. Immunofluorescence is pivotal for diagnosing AIBDs. Tissue-bound and/or circulating autoantibodies and the patterns of their binding to specific antigens can be demonstrated by direct and indirect immunofluorescence, respectively.

Aim of the Work: To evaluate the performance characteristics of anti-skin antibodies IgG by indirect immunofluorescence (IIF) in the diagnosis of autoimmune bullous diseases.

Subjects and Methods: Twenty-five patients with AIBDs including 14 pemphigus vulgaris (PV) patients, 5 pemphigus foliaceus (PF) patients and 6 bullous pemphigoid (BP) patients were studied, and compared with 10 patients with non-bullous skin diseases and 10 healthy subjects as controls. ASA-IgG were investigated by using IIF technique.

Results: Positive anti-skin antibodies (ASA)-IgG IIF test was demonstrated in 92% of AIBD patients and in 20% of patients with non-bullous skin diseases. The test was negative in all ten healthy subjects. Thus, ASA-IgG were significantly positive in AIBDs than both non-bullous diseases patients and healthy control group. Two patterns of ASA-IgG binding to the corresponding antigens were seen; intercellular substance (ICS) pattern with serum samples from PV and PF patients that diagnose intraepidermal blistering diseases. Linear basement membrane zone (BMZ) pattern by serum samples obtained from BP patients that diagnose subepidermal blistering diseases.

Conclusions: IIF assay is an accurate screening test for detection of circulating ASA-IgG that categorizes autoimmune bullous disorders into two major subtypes: pemphigus and pemphigoid based on antibody deposits staining patterns either ICS or linear BMZ pattern, respectively.

Keywords: Autoimmune Bullous Diseases, Pemphigus Vulgaris, Anti-Skin Antibodies IgG, Indirect Immunofluorescence.

I. INTRODUCTION

Autoimmune bullous diseases are acquired heterogenous disorders of skin and mucosae, which are caused by autoantibodies, commonly of IgG and/or IgA classes, against structural proteins mediating cell–cell and cell–matrix adhesions. The interactions between auto-antibodies and these structural proteins cause loss of adhesion and blister formation at different levels. Thus, AIBDs are divided into intraepidermal (pemphigus) and subepidermal (pemphigoid) blistering diseases. Chronic blisters and erosions are the main clinical characteristic

of AIBDs. Histopathology demonstrates the location of blister formation [1, 2].

In pemphigus, anti-skin antibodies bind to protein antigens in the cell surface of keratinocytes. Desmoglein 1 (Dsg1) and desmoglein 3 (Dsg3), the major autoantigens in pemphigus, are transmembrane glycoprotein components of desmosomes; the structure complex responsible for adhesion between epidermal cells. Pemphigus vulgaris (PV), the most common subtype in pemphigus, is characterized by auto-antibodies mainly targeting Dsg3, while the autoantigen in pemphigus foliaceus (PF), the second most common subtype, is Dsg1. In the other major category of AIBDs (pemphigoid diseases), auto-antibodies are directed against distinct structural proteins of hemidesmosomes and anchoring fibrils in the dermal–epidermal junction (DEJ) that mediate cell-matrix attachment. Bullous pemphigoid is the most common subtype in pemphigoid diseases, in which autoantibodies are directed against two principle hemidesmosomal proteins: trans-membrane bullous pemphigoid antigen 2 (BPAg2) and intracytoplasmic bullous pemphigoid antigen 1 (BPAg1) [3,4].

For the diagnosis of AIBDs, in addition to clinical manifestations and histopathological examination, it is mandatory to detect immunologically the tissue-bound and/or circulatory IgG and/or IgA autoantibodies. Tissue-bound antibodies are demonstrated by direct immunofluorescence on perilesional skin biopsy. Detection of autoantibodies in AIBDs, characterization of their microscopic binding patterns by direct or indirect immune-fluorescence, and investigating their target specific antigens by highly sensitive and specific ELISA systems or immunoblotting are obligate requirements for diagnosing AIBDs [5-7].

Several studies documented the sensitivity of indirect immunofluorescence in patients with PV, PF, and BP as they are the most common subtypes of AIBDs with varied results for each disease [8-11].

In this study, we attempted to investigate the sensitivity and specificity of IIF technique for the detection of anti-skin antibodies IgG in AIBDs.

II. METHODS AND MATERIAL

A. Subjects

Serum samples were collected from 35 patients from the Dermatology Department at the Main University Hospital, Alexandria Faculty of medicine and divided into two groups. Twenty-five patients suffering from AIBDs (group I) which included 14 patients diagnosed as PV, 5 patients diagnosed as PF, and 6 patients with BP. The diagnosis of patients with AIBDs was based on clinical and histological diagnostic criteria. Ten patients with non-bullous skin diseases (group II) included 6 patients with psoriasis and 4 cases suffering from drug reaction. In addition to, ten apparently healthy subjects (group III) with matched age and sex were included as a control group. All collected sera were stored at -80 ^{o}C until assayed.

The study was approved by the ethics committee of the Faculty of Medicine, Alexandria University and informed written consents were taken from all subjects enrolled in this study.

B. ASA-IgG indirect immunofluorescence

Anti-skin antibodies were examined using a commercial kit (anti-skin antibodies indirect immunofluorescence monkey oesophagus as a substrate by BioSystems, Spain), REF 44560, 44562, and 44563. FITC-conjugated anti-human IgG was used as secondary antibodies.

III. RESULTS AND DISCUSSION

A. RESULTS

Demographic data showed no significant difference between the studied groups as regards age and gender.

ASA-IgG IIF test was positive in 23 of the 25 AIBD patients (92%) and in 2 of 10 patients with non-bullous skin diseases (20%). The test was negative in all ten healthy controls. ASA-IgG were significantly positive in AIBDs than both non-bullous diseases patients ($p=0.001$) and healthy control group ($p=0.001$) “Fig. 1”

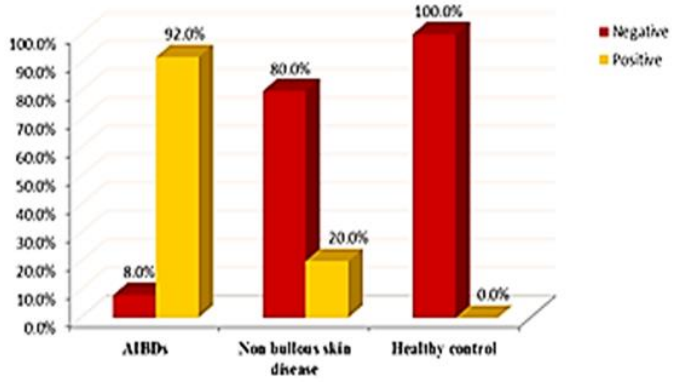

Figure 1: Anti-skin antibodies immunoglobulin G indirect immunofluorescence in the studied groups

Performance characteristics of ASA-IgG by IIF were calculated "Table 1".

Table 1: Diagnostic performance of anti-skin antibodies immunoglobulin G indirect immunofluorescence in autoimmune bullous diseases.

	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
ASA-IgG IIF	92.0%	90.0%	92.0%	90.0%	91.1%

The presence of anti-skin autoantibodies in serum samples were demonstrated by the appearance of apple-green fluorescence on the corresponding histologic structures. Two different patterns were detected due to the localization of the specific target antigens. Intercellular substance (ICS) staining pattern, a net-like pattern, is characteristic in pemphigus patients "Fig. 2". While, in pemphigoid diseases (BP), a linear fluorescent staining along the basement membrane zone (linear BMZ pattern) is seen "Fig. 3". All PV (100%) and 80% (4/5) of PF patients showed the same ICS pattern with no significant difference (p=0.574). Five of six BP patients (83.3%) were positive with linear BMZ pattern that was significantly different from pemphigus patients (p=0.001) "Fig. 4".

Additionally, sera from two patients with non-bullous skin diseases, who were diagnosed as psoriasis patients, showed the ICS pattern.

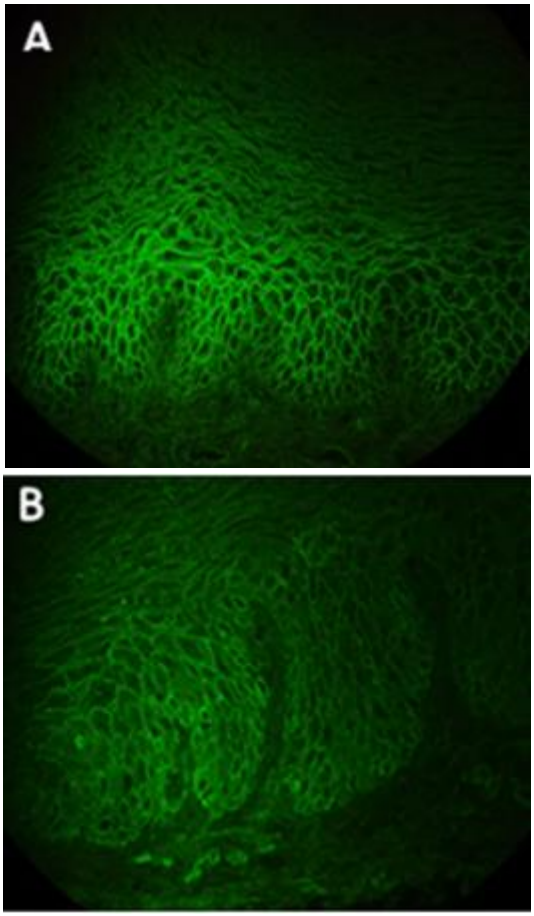

Figure 2: Intercellular substance staining pattern of anti-skin antibodies immunoglobulin G deposits by indirect immunofluorescence. A) Pemphigus vulgaris patient. B) Pemphigus foliaceus patient

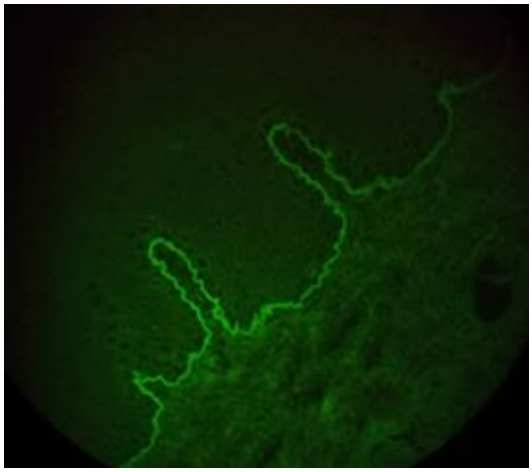

Figure 3: linear basement membrane zone staining pattern of anti-skin antibodies immunoglobulin G deposits of bullous pemphigoid patient.

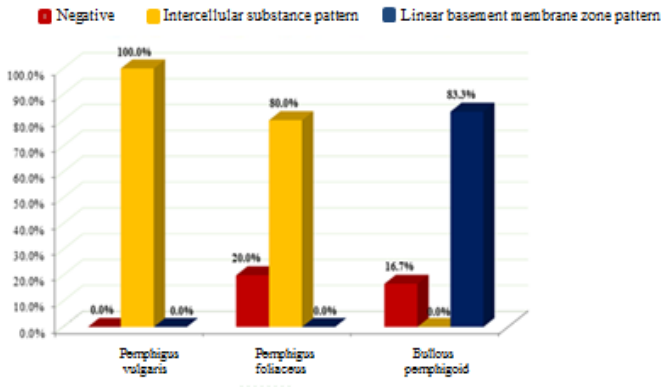

Figure 4: Different anti-skin antibodies binding staining patterns in group I

B. DISSCUSION

The serological hallmark of AIBDs is the detection of (IgG and/or IgA) autoantibodies directed against either the desmosomal protein antigens (Dsg1 and Dsg3) responsible for cell-cell adhesion in stratified squamous epithelia in pemphigus patients or against structural protein antigens in DEJ in stratified epithelium causing subepidermal blistering diseases. Subsequently, loss of adhesion, blister formation and extensive erosions occur in the skin and/or mucous membranes [5, 6].

The present study demonstrated the accuracy (91.1%) of anti-skin antibodies IgG IIF test in the diagnosis of AIBDs. The diagnostic sensitivity of ASA-IgG IIF was 100% for PV, 80% for PF with an overall sensitivity of 94.7% for pemphigus.

Similarly, previous studies documented that the sensitivity of this test in patients with pemphigus was ranging from 84 to 90%. The sensitivity of the test varies according to the used substrate. The highest reported sensitivities were by using monkey esophagus as a substrate. Zhou et al,[8] Wang et al, [12] and Aksu et al, [13] reported a diagnostic sensitivity of 84.8% for pemphigus, 87.5% for PV and 89% for PV, respectively. According to Harman et al,[10] for pemphigus patients, the overall sensitivity of Anti-ICS IIF was 83% on human skin (HS) and 90% on monkey esophagus (MO). In PF patients the sensitivity of IIF was 100% on HS and 67% on MO. In contrast, IIF sensitivity was greatest on MO in PV, 100% compared with 75% on HS. Also, Ng et al, [14] documented a total sensitivity of 86% for pemphigus by using both human skin and monkey esophagus substrates. In contrast, Marinović et al, [11] mentioned a low sensitivity of 73.3% for PV (14:19) and all three PF cases were positive by using only human skin substrate.

In this study, anti-skin antibodies IgG IIF showed a sensitivity of 83.3% (5/6) for BP. Sárdy et al, [9] and Barnadas et al, [15] reported sensitivities of 73.2% (229/313) and 78.2% (18/23), respectively.

No substrate is generally sensitive for all subtypes of AIBDs. Indirect IF on monkey esophagus (in which Dsg 3 is strongly expressed in the epithelium) tends to be more sensitive in patients with PV. In contrast, IIF on human skin or guinea pig esophagus (in which Dsg 1 is strongly expressed in the epithelium) shows more sensitivity in testing for serum antibodies in patients with PF. Whereas for the subepidermal autoimmune blistering diseases, monkey esophagus and salt split normal human skin are the most commonly used substrates; the latter is the preferred substrate as normal human skin that has been split with 1 M sodium chloride solution at the level of lamina lucida shows a higher sensitivity exposing different antigens in the BMZ. IIF on salt split skin (SSS) allows improved localization of the target antigen in the BMZ [5, 7, 16].

The specificities of the test were 90% for pemphigus and 100% for BP. Similarly, Zhou et al, [8] reported a specificity of 91.8% in pemphigus, whereas in studies performed on BP patients, Sárdy et al, [9] and Barnadas et al, [15] reported specificities of 97.1% and 96%, respectively. On contrary, Wang et al, [12] obtained a lower specificity of 72.1% in pemphigus diagnosis.

Noteworthy, we noticed no difference in the ICS staining pattern in PV and PF and found that IIF, performed only on one substrate, cannot differentiate between these two pemphigus subtypes. Harman et al, [10] suggested that the use of two substrates for IIF screening: one rich in Dsg1, such as HS, and the other rich in Dsg3, such as MO. This combination of substrates should not only increase the sensitivity of detecting pemphigus antibodies, but also will aid in the differentiation of PV from PF. As in his study, serum samples from PF patients showed higher titers on human skin, while that from PV patients revealed higher titers on monkey esophagus.

In contrast to our observation, Jarząbek-Chorzelska et al, [17] mentioned that the ICS pattern on one epithelial substrate (monkey esophagus or human esophagus) enabled differentiation between PV and PF. According to that previous study, PV stained the whole epithelium, but PF stained only the upper epithelial layers reflecting the distribution of Dsg3 (located in all histological layers) and Dsg1 (located only in the upper histological layers) in the mucosae with stratified squamous epithelium.

Another finding in our study was that we detected anti-intercellular substance autoantibodies in sera obtained from two of 14 patients with PV (14.2%) while they were in clinical remission.

Similarly, a previous study by Barnadas et al, [18] in which anti-epithelial antibodies were detected in serum samples from PV patients in remission. Also, Kamiya et al, [19] using ELISA assay, found PV patients in remission with elevated index values of anti-Dsg3 >100. Furthermore in a study by Daneshpazhooh et al, [20] eighty-nine PV patients in complete clinical remission were tested to identify immunologic predictors (anti-Dsg1 and 3 antibodies and direct immunofluorescence) for relapse. DIF was positive in 44 of 89 patients (49.5%), anti-Dsg 3 antibodies were detected in 18 of 46 patients (39.1%), and anti-Dsg 1 antibodies were detected in 4 of 46 patients (8.7%).

This could be explained by the presence of nonpathogenic antibodies in sera of pemphigus patients in remission. Possible mechanisms are considered to explain the causes of loss of the pathogenicity of the serum anti-skin antibodies [21]. Firstly, the possibility of that non-pathogenic autoantibodies react with precursor fragment on immature Dsg, which is present in the endoplasmic reticulum [22-24]. Also, pathogenicity depends on extracellular domains of Dsg. Several studies have suggested that pathogenic autoantibodies react with N-terminal domains of mature Dsg (EC1 or EC2 domain), and autoantibodies to EC3-EC5 are non-pathogenic [25-27]. Lastly, pathogenicity depends on IgG subclasses (IgG1-IgG4) in pemphigus patients. IgG4 subclass predominates in active disease, while IgG1 subclass is the predominant in patients in remission [28, 29].

Regarding the two psoriasis patients whose sera showed ICS staining pattern, they should undergo further investigations for detection of anti-desmoglein 1 and 3 antibodies by ELISA and to be followed up for any clinical manifestations of pemphigus. It may be a preclinical stage for pemphigus as a coincidence between psoriasis and autoimmune bullous diseases was mentioned in previous studies [30, 31].

Kwon et al, [30] reported pemphigus foliaceus developed on pre-existing psoriasis in six cases. The period between appearance of psoriasis and pemphigus foliaceus varied from 8 months to 52 years. Ohata et al, [31] pointed out the frequent occurrence of psoriasis in patients with autoimmune bullous diseases. Psoriasis onset preceded AIBD onset in most patients with a mean duration between psoriasis and AIBD onset of 14.6 years.

This current study was limited by the small number of bullous pemphigoid patients representing subepidermal autoimmune blistering diseases. Additionally, follow up sera were unavailable and antibody titers for patients' samples were not done, consequently the correlation of diagnostic data with disease severity and disease course was not possible.

IV. CONCLUSION

In conclusion, this study provided that anti-skin antibodies IgG IIF, using monkey esophagus as a substrate, can be used as a complementary serological screening test for the diagnosis of autoimmune bullous diseases.

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