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Comparison of Diagnostic Value of Indirect Immunofluorescence Assay and Desmoglein ELISA in the Diagnosis of Pemphigus

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SUMMARY Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are autoimmune blistering diseases characterized by intraepidermal separation as the result of autoantibodies directed to desmoglein 1 and desmoglein 3, adhesion molecules that have a pathogenic role in blister formation. Both PV and PF are diagnosed according to clinical picture, histopathologic, immunopathologic and molecular biologic features. In the present study, the value of indirect immunofluorescence (IIF) and enzyme linked immunosorbent assay (ELISA) for desmoglein 1 (Dsg 1) and desmoglein 3 (Dsg 3) at baseline visit was compared. The study was performed as a retrospective study that included 22 patients, 19 of them with PV and three with PF. Patient sera were tested with IIF and Dsg 1 and Dsg 3 ELISA. In the group of 19 PV patients, 12 patients had positive IIF, Dsg 3 and Dsg 1 ELISA; two had positive IIF and positive anti Dsg 3 but negative anti Dsg 1; three had negative IIF but positive both Dsg 1 and Dsg 3 antibodies; and two had negative IIF and Dsg 1 but positive Dsg 3 antibodies. In the group of PF patients, all three patients had positive IIF, positive Dsg 1 ELISA and negative Dsg 3 ELISA. Results of our study supported previous reports confirming Dsg 1 and Dsg 3 ELISA to be a sensitive and specific tool for the diagnosis of PV and PF.

KEY WORDS: pemphigus vulgaris, pemphigus foliaceus, indirect immunofluorescence, anti desmoglein ELISA

INTRODUCTION

Pemphigus is a group of autoimmune blistering diseases characterized with intraepidermal blisters and erosions on the skin and/or mucous membranes. Two main forms of pemphigus are pemphigus vulgaris (PV) and pemphigus foliaceus (PF), each of them having few subtypes (1). These two major forms of pemphigus are distinguishable from each other according to clinical picture, histopathologic, immunopathologic and molecular biologic features (2). In PF blisters and

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erosions appear most often on the face and trunk without involvement of oral mucosa, while histopathology shows subcorneal acantholytic bullae. In PV patients blisters present as flaccid bullae and erosions on the skin, almost always associated and often preceded by oral mucosal lesion, while histopathology shows suprabasilar acantholytic bullae in lower epidermis (2,3). Blisters in PV and PF result from the presence of circulating IgG autoantibodies directed to desmoglein 3 (Dsg 3) and desmoglein 1 (Dsg 1). Both Dsg 1 and Dsg 3 are full length transmembrane proteins that belong to the cadherin family, are expressed in stratified squamous epithelia and play pathogenic roles in blister formation in PF and PV, respectively (2,3). Patients with PV whose disease is restricted to mucosa mostly have autoantibodies directed against Dsg 3 and patients with lesions on the skin and mucous membrane have both autoantiboides directed against Dsg 1 and Dsg 3. Autoantibodies directed to desmoglein 1 are characteristically found in PF patients (4). The diagnosis of the pemphigus group of diseases is based on clinical picture and history, histopathologic diagnosis, and direct and indirect immunofluorescence (IIF). Since the introduction of commercially available enzyme-linked immunosorbent assay (ELISA) kits for Dsg 3 and Dsg 1, these tests have been used as an additional diagnostic tool for diagnosing PV and PF (5). In most cases, the specificity of ELISA results reflects the clinical phenotype of the disease (2). ELISA index also provides a valuable tool to monitor disease activity because recent studies show that ELISA index values fluctuate in parallel with disease activity (6-8).

AIM OF THE STUDY

The aim of the study was to compare diagnostic value of IIF and Dsg 1 and Dsg 3 ELISA as a tool in diagnosing and distinguishing between PV and PF. The usefulness of Dsg 1 and Dsg 3 ELISA in the diagnosis of pemphigus in Croatian patients has not yet been reported. This study is part of the scientific project entitled Autoimmune Blistering Diseases in Croatia, the main goal of which is to give an overview of various aspects of these diseases in the Republic of Croatia.

PATIENTS AND METHODS

Patients

This retrospective study included 22 patients with PV and PF referred to our Department between January 1, 2008 and June 30, 2009. In this study, only results of IIF and Dsg 1 and Dsg 3 ELISA at baseline visit were used. Prior to inclusion in the study, patients were diagnosed with PV or PF according to clinical picture, histopathologic and/or immunofluorescence features. The study was approved by the Ethics Committee of the Zagreb University Hospital Center and School of Medicine.

Methods

Indirect immunofluorescence assay

Normal human skin was used as a substrate for IIF assay. Patient sera were diluted up to 1:320 for IgG antibodies.

ELISA

The sera of all 22 patients were tested using Dsg 1 and Dsg 3 ELISA kits (MESACUP Desmoglein test, MBL, Nagoya, Japan) according to the manufacturer's instructions. The absorbance of each well was read at 450 nm by an automated plate reader (Asys Hitech Model Expert Plus). Positive and negative calibrators provided in the kit were included in each run. On evaluation of the sensitivity and specificity of ELISA, the cut-off values of 14 U/mL for Dsg 1 and 7 U/mL for Dsg 3 were considered.

Statistics

On statistical analysis, the STATISTICA Version 8 (StatSoft, Inc.) software was used.

RESULTS

Results are presented in tables and figures.
The analysis included sera from 22 patients (Table 1). PV was previously diagnosed in 19 and PF in three patients. All three patients with PF diagnosed at our Department were females. In the PV group, there were 15 female and four male patients (female to male ratio, 3.75:1).

In the group of PF patients, all three patients had positive IIF, positive anti Dsg 1 ELISA and negative anti Dsg 3 ELISA.

In the group of 19 patients with PV, 12 (62.15%) patients had positive IIF, Dsg 3 and Dsg 1 ELISA (all parameters); two (10.5%) of them had positive IIF and positive anti Dsg 3 but negative anti Dsg 1; three (15.78%) had negative IIF but positive both Dsg 1 and Dsg 3 antibodies; and two (10.5%) had negative IIF and Dsg 1 but positive Dsg 3 antibodies (Fig. 1).

Table 1. Results of indirect immunofluorescence (IIF), anti Dsg 1 and anti Dsg 3 ELISA

No.	Sex (male/ female)	Diagnosis	IIF	Dsg 1	Dsg 3
1	F	PV	+ 1:40	+ 133	+ 171
2	M	PV	-	+ 19	+120
3	F	PV	+ 1:80	+ 112	+ 185
4	M	PV	> 1:320	+ 203	+ 414
5	F	PV	+ 1:40	+ 124	+ 147
6	F	PV	+ 1:80	+ 205	+ 86
7	F	PV	-	-	+ 97
8	M	PV	+ 1:320	+ 173	+ 174
9	F	PV	> 1:320	+ 88	+ 524
10	F	PV	+ 1:80	+ 72	+ 504
11	F	PV	-	-	+ 161
12	F	PV	-	+ 37	+ 9
13	F	PV	+ 1:320	+ 116	+ 173
14	F	PV	+ 1:20	-	+ 162
15	F	PV	-	+ 74	+ 26
16	F	PV	+ 320	+ 82	+ 191
17	M	PV	+ 1:40	-	+ 204
18	F	PV	+ 1:20	+ 15	+ 198
19	F	PV	+ 1:80	+ 192	+ 232
20	F	PF	+ 1:80	+ 176	-
21	F	PF	+ 1:320	+ 198	-
22	F	PF	+ 1:80	+ 294	-

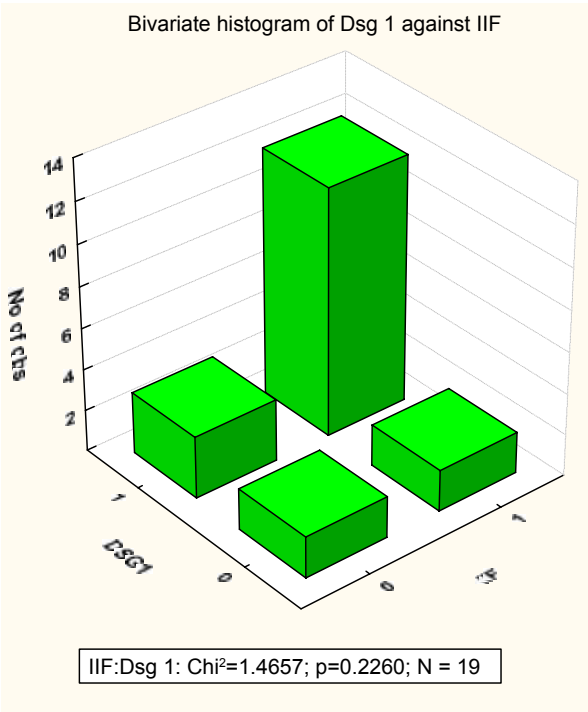

Figure 1. Dsg 1 ELISA and IIF parameters in pemphigus vulgaris patients.

Table 2. Comparison between IIF and Dsg 1 ELISA in pemphigus vulgaris patients

	IIF		
Dsg 1	0	1	
0	2	2	4 (21.1%)
1	3	12	15 (78.9%)
	5 (26.3%)	14 (73.7%)	19

Using χ^2-test, the distribution of positive results between Dsg 1 ELISA and IIF assay was not statistically significant (P=0.2260) (Table 2).

Thus, it was not possible to use χ^2-test for comparing Dsg 3 and IIF because Dsg 3 results were positive in all patients (Table 3, Fig. 2).

Table 3. Comparison between IIF and Dsg 3 ELISA in pemphigus vulgaris patients

	IIF		
DSG 3	0	1	
1	5	14	19 (100.0%)
	5 (26.3%)	14 (73.7%)	19

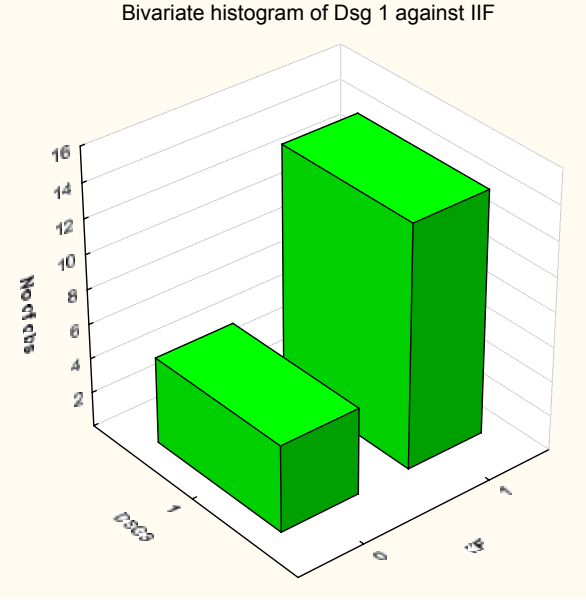

Figure 2. Dsg 3 ELISA and IIF parameters in pemphigus vulgaris patients.

DISCUSSION

The aim of our study was to compare IIF assay and Dsg 1 and Dsg 3 ELISA as a tool in the diagnosis of PV and PF in Croatian patients. In the PF group, all three patients had positive IIF and Dsg 1 and negative Dsg 3, which is comparable with the current knowledge, yet the group was too small for statistical analysis (2-4). Any comparison of their results with the others or to make any valid conclusion, a larger patient group and additional studies are needed.

In the PV group, five of 19 (26.3%) patients had negative IIF and positive Dsg 3 and/or Dsg 1 ELISA. Although IIF is useful for identifying antibodies, there is the possibility of false negative results due to substrate sensitivity, technical error, and also very rarely prozone phenomenon (9). The possible reason for some negative IIF results was the fact that we used human skin as a substrate for IIF assay, and not monkey esophagus (in which is Dsg 3 is strongly expressed in the epithelium) which is reported as a more sensitive substrate for pemphigus patients (2).

In the group of PV patients, 15 of 19 (78.9%) patients had positive anti Dsg 1 antibodies and all patients (100%) had positive anti Dsg 3 antibodies, which is comparable with the study by Atzori *et al*. (10). Our results are also comparable with the study by Abasq *et al*., who report that their results of ELISA at baseline confirmed the previously reported correlation between clinical phenotype of patients and recognition of Dsg 1 and Dsg 3 in serum. However, they also found the Dsg 1 ELISA antibody values to more closely correlate than anti Dsg 3 antibodies during follow up (11).

In our group of patients, clinical phenotype was related to the antibody profile. There are occasional reports of cases with discordant clinical phenotype and antibody profile (12-14). The present study supported previous reports on ELISA for anti Dsg 1 and Dsg 3 antibodies to be a sensitive and specific tool for the diagnosis of pemphigus (9). According to the authors and our latest experience (unpublished data), it can also serve as a predictive means to monitor disease activity (2,5).

Unlike IIF, one of the advantages of ELISA is that it does not require specifically skilled observer. It also reflects disease activity in early stages better than IIF (7).

CONCLUSION

Dsg 1 and Dsg 3 ELISA is a sensitive tool for diagnosing pemphigus, in which positive Dsg 3 antibodies are indicative of PV, regardless of the associated Dsg 1 result. Positive Dsg 1 with negative Dsg 3 is indicative of the diagnosis of PF. ELISA is not only a sensitive diagnostic tool, as it can also serve as a predictive means to monitor disease activity. There is a need of more patients to be included in further studies comparing the two methods, but also to assess the follow up index values of Dsg 1 and Dsg 3 ELISA.

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