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Salivary Samples for the Diagnosis of *Pemphigus vulgaris* Using the BIOCHIP Approach: a Pilot Study

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Abstract. *Pemphigus vulgaris (PV) is a rare autoimmune intraepithelial blistering skin disease characterized by the presence of circulating autoantibodies against desmoglein 3 (DSG3) and desmoglein 1 (DSG1), resulting in loss of the normal epithelial cell-to-cell adhesion, through a process called acantholysis. In recent years, a BIOCHIP-based indirect immunofluorescence technique for the determination of anti-DSG3 and anti-DSG1 autoantibodies has been described. Even though, the use of saliva anti-DSG3 and anti-DSG1 ELISA for the diagnosis of PV has been already reported, there are no studies concerning the utilization of saliva by the BIOCHIP approach. In the present pilot study, ELISA and BIOCHIP were performed, using salivary and serum samples from the same patients to investigate if the detection of anti-desmoglein autoantibodies in salivary samples by BIOCHIP could be used as a test for the diagnosis of PV. There was a strong correlation between ELISA and BIOCHIP results both for anti-DSG3 and anti-DSG1 serum autoantibodies. Autoantibodies to DSG3 were detected in 8 out of 8 salivary samples by ELISA and in 6 out of 8 salivary samples by the BIOCHIP approach. Autoantibodies to DSG1 were negative in all salivary samples using both ELISA and BIOCHIP. There were no positive results in the negative control group. In conclusion, the results of this pilot study indicate lack of correlation between serum and salivary results using both ELISA and BIOCHIP, indicating that saliva may not be the ideal substrate for the laboratory diagnosis of PV using these approaches.*

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Key Words: Pemphigus vulgaris, saliva, desmoglein 1, desmoglein 3, BIOCHIP-based indirect immunofluorescence technique.

Pemphigus vulgaris (PV) is a rare autoimmune intraepithelial blistering skin disease characterized by the presence of circulating autoantibodies directed against desmoglein 3 (DSG3) and desmoglein 1 (DSG1), resulting in loss of the normal epithelial cell-to-cell adhesion, through a process called acantholysis (1). The diagnosis of PV is generally based on clinical features, histology and immunological tests, notably direct and indirect immunofluorescence and enzyme linked immunoassorbent assay (ELISA) (2). In recent years, a BIOCHIP-based indirect immunofluorescence technique for the determination of anti-DSG3 and anti-DSG1 autoantibodies has been described (3, 4). Today, serum anti-DSG3 and anti-DSG1 ELISA is regularly used for the diagnosis and follow up of pemphigus (5, 6). Saliva may be also used for a non-invasive diagnosis of this autoimmune skin condition. The use of saliva anti-DSG3 and anti-DSG1 ELISA for the diagnosis of PV has been already reported (7, 8). In the present pilot study, we performed both ELISA and BIOCHIP using salivary and serum samples from the same patients to investigate if the detection of anti-desmogleins autoantibodies in salivary samples by BIOCHIP could be used as test for the diagnosis of PV.

Patients and Methods

The study comprised 8 caucasian patients with PV, 3 males and 5 females (Table I). The diagnosis of PV was based on clinical features, histology and immunopathological findings, notably positive direct immunofluorescence, serum detection of anti-DSG3 and anti-DSG1 autoantibodies by ELISA and serum BIOCHIP. Serum and salivary samples from 2 normal healthy individuals, 1 patient with *epidermolysis bullosa acquisita* and 2 patients with *bullous pemphigoid* were used for negative controls (Table II).

For the detection of autoantibodies, an ELISA assay was employed using recombinant proteins DSG1 and DSG3 (MBL, Nagoya, Japan), made of the entire extracellular domain of DSG1 and DSG3 respectively and produced by Amagai *et al*. (9) with cut off values of 20 U/ml for DSG1 and DSG3. For the detection of autoantibodies by BIOCHIP Technology recombinant DSG1 and DSG3 expressing human cells (HEK293 cells) have been used as substrates (3). They are applied to thin glass slides, mechanically cut into millimeter-sized fragments (BIOCHIPs) and then applied

Table I. *Results at the time of diagnosis. ELISA values are expressed as unit/ml. BIOCHIP results are expressed as positive or negative.*

Patient no.	Gender	Disease	ELISA DSG1 serum	ELISA DSG3 serum	BIOCHIP DSG1 serum	BIOCHIP DSG3 serum	ELISA DSG1 saliva	ELISA DSG3 saliva	BIOCHIP DSG1 saliva	BIOCHIP DSG3 saliva
1	F	PV	2,85	102,23	Neg	Pos	neg	neg	Neg	Neg
2	M	PV	52,09	174,68	Pos	Pos	0,36	0,33	Neg	Pos
3	F	PV	2,72	158,26	Neg	Pos	6,26	155,57	Neg	Pos
4	M	PV	1,02	20,21	Neg	Pos	1,39	2,75	Neg	Pos
5	M	PV	17,45	162,17	Neg	Pos	9,19	123,56	Neg	Pos
6	F	PV	1,76	106,28	Neg	Pos	1,39	38,95	Neg	Neg
7	F	PV	5,01	204,73	Neg	Pos	4,18	82,54	Neg	Pos
8	F	PV	4,08	223,64	Neg	Pos	3,43	209,29	Neg	Pos

F, Female; M, male; PV, *pemphigus vulgaris*; Neg, negative; Pos, positive.

Table II. *Results at the time of diagnosis. ELISA values are expressed as unit/ml. BIOCHIP results are expressed as positive or negative.*

Patient no.	Gender	Disease	ELISA DSG1 serum	ELISA DSG3 serum	BIOCHIP DSG1 serum	BIOCHIP DSG3 serum	ELISA DSG1 saliva	ELISA DSG3 saliva	BIOCHIP DSG1 saliva	BIOCHIP DSG3 saliva
1	M	C	0	0	Neg	Neg	0	0	Neg	Neg
2	F	C	0	0	Neg	Neg	0	0	Neg	Neg
3	F	EBA	2.42	0	Neg	Neg	14.88	15.51	Neg	Neg
4	F	BP	0	0	Neg	Neg	0	0	Neg	Neg
5	F	BP	4.53	2.35	Neg	Neg	6.62	6.62	Neg	Neg

F, Female; M, male; C, Control (healthy individual); BP, bullous pemphigoid; EBA, epidermolysis bullosa acquisita; Neg, negative; Pos, positive.

automatically onto microscopes slides (3, 6, 10). The Titerplane Technique, consisting of two consecutive incubation steps (in the first the substrates are incubated with diluted (1/10) patient serum samples, in the second the attached autoantibodies are stained with fluorescein-labeled anti-human antibodies), was the procedure used. Evaluation of results was made by conventional fluorescence microscopy.

Results

All 8 PV patients tested positive both by ELISA and BIOCHIP for serum anti-DSG3 autoantibodies. Only 1 patient tested positive by ELISA and BIOCHIP for serum anti-DSG1 autoantibodies. There was a perfect correlation between ELISA and BIOCHIP results both for anti-DSG3 and anti-DSG1 serum autoantibodies. We did not observe the same correlation between ELISA and BIOCHIP results when using salivary samples. Autoantibodies to DSG3 were detected by ELISA in 8 out of 8 patient's salivary samples and by the BIOCHIP approach in 6 out of 8. Autoantibodies to DSG1 were negative in all salivary samples using both ELISA and BIOCHIP (Table I). There were no positive results in the negative control group (Table II).

Discussion

Saliva is a biofluid that can be simply obtained from patients and easily repeated. Serum components such as autoantibodies could be transported to saliva through capillary walls of the salivary glands. Being a non-invasive test, the detection of anti-DSG3 and anti-DSG1 autoantibodies in salivary samples could be used as a safe test for the diagnosis of PV. The aim of our study was to investigate the diagnostic accuracy of ELISA and BIOCHIP for the diagnosis of PV. While using serum samples, a strong association between ELISA and BIOCHIP results has been demonstrated (4), the present study doesn't show a correlation of results between these two methods using salivary samples. Furthermore, despite some authors (7, 8) describing high sensitivity and specificity of ELISA assay to detect anti-desmoglein autoantibodies in saliva, in our opinion saliva cannot be considered a good substitute of serum for the diagnosis of this autoimmune bullous disease. In our study group, we did not find a strict correlation between serum and salivary autoantibodies detection. In conclusion, the results of this pilot study indicate a lack of correlation between

serum and salivary results by both ELISA and BIOCHIP tests indicating that saliva may not be the ideal substrate for the laboratory diagnosis of PV using these approaches.

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