Acta Dermatovenerol Croat 2017;25(3):202-209 CLINICAL ARTICLE

The Value of the BIOCHIP Mosaic-based Indirect Immunofluorescence Technique in the Diagnosis of Pemphigus and Bullous Pemphigoid in Turkish Patients

Birgül Özkesici[1], Derya Mutlu[2], Levent Dönmez[3], Soner Uzun[1]

[1]Department of Dermatology, Akdeniz University Faculty of Medicine, Antalya, Turkey; [2]Department of Microbiology-Clinical Microbiology, Akdeniz University Faculty of Medicine, Antalya, Turkey; [3]Department of Public Health, Akdeniz University Faculty of Medicine, Antalya, Turkey

*This paper was presented at the 24th EADV Congress, Copenhagen-Denmark, 7-11 October, 2015.

Financial information:

This work was supported by The Scientific Research Projects Coordination Unit of Akdeniz University. Project Number: 2014.04.0103.008

Corresponding author:

Prof. Soner Uzun, MD

Department of Dermatology and Venerology

Akdeniz University, School of Medicine

07058 Antalya

Turkey

sonuzun@hotmail.com

Received: October 30, 2016

Accepted: July 21, 2017

ABSTRACT Autoimmune bullous diseases (ABD) are a rarely seen group of diseases, of which pemphigus and bullous pemphigoid (BP) are the major groups. Diagnosis is generally based on the combination of clinical features, histopathologic and immunofluorescence (IF) findings, and/or enzyme-linked immunosorbent assay (ELISA). Aims of the work were to determine the value of the innovative BIOCHIP mosaic-based indirect IF technique in the diagnosis of pemphigus and BP in Turkish patients. A total of 63 patients (45 pemphigus and 18 BP) in the active phase of the disease alongside 35 healthy controls were included in the study. All sera from patients and controls were tested using the BIOCHIP technique, and the results were compared with direct IF and/or ELISA. The sensitivity and specificity of this new technique were calculated for validity. The sensitivity and specificity of BIOCHIP in the diagnosis of pemphigus was found to be 91.1% and 97.1%, respectively. In detection of anti-Dsg1 and anti-Dsg3 autoantibodies, the correlation between BIOCHIP and ELISA was statistically significant ($P<0.01$). The sensitivity and specificity of BIOCHIP in the diagnosis of BP was found to be 94.4% and 94.3%, respectively. In detection of anti-BP180 autoantibodies, the correlation between the BIOCHIP and ELISA was statistically significant ($P<0.01$). The main limitations are the relatively low number of samples and testing with only one dilution. Direct IF was not performed in all patients, and the low rate of DIF positivity also can be a bias in comparison with BIOCHIP. The new BIOCHIP technique is a highly sensitive and specific tool in the diagnosis of pemphigus and BP.

KEY WORDS: BIOCHIP, pemphigus, bullous pemphigoid, immunofluorescence, ELISA

INTRODUCTION

Autoimmune bullous diseases (ABD) are potentially life threatening diseases characterized by autoantibodies formed against the structural proteins of the epidermis or the dermal-epidermal junction (1-3). Currently, demonstration of such antibodies is the gold standard in the diagnosis of this group of diseases. The methods that are used for this purpose are: direct immunofluorescence (DIF), indirect im-

Acta Dermatovenerol Croat
2017;25(3):202-209

munofluorescence (IIF), enzyme-linked immunosorbent assay (ELISA), immunoprecipitation and immunoblotting (4,5). In routine examinations, DIF is the most commonly used among these tests. Although it is noted to be quite sensitive in reported studies, this sensitivity can show variability depending on the centers in which it is performed. Similarly, ELISA is highly sensitive and specific in the diagnosis of this group of diseases. The most important advantage of ELISA is determining antibody titers as a quantitative test (6). In recent years, the BIOCHIP mosaic-based IIF technique has been developed as a new method for the routine diagnosis of ABD (7-11). The presence of multiple antigenic structures on a BIOCHIP slide enables a differential diagnosis among the ABD subtypes from a single serum sample.

In this study, our goal was to determine the value of this new method in the diagnosis of pemphigus and bullous pemphigoid (BP) by using the BIOCHIP mosaic-based IIF technique. In addition, we also evaluated whether the BIOCHIP technique is compatible or not with those routine diagnostic methods that are currently accepted as the gold standard diagnostic tools for this group of diseases.

PATIENTS AND METHODS

Patients

In this study, a total of 63 patients (45 with pemphigus and 18 with BP) in the active phase of the disease were included, alongside 35 healthy controls. The study was conducted prospectively. Healthy volunteers with autoimmune diseases and under immunosuppressive treatment were not included in the control group. The patients in the active phase of the disease, either at first presentation or with relapse, were included the study. The histopathologic examination of all patients was compatible with pemphigus or BP, and the diagnosis of pemphigus or BP was confirmed by DIF and/or ELISA tests. DIF was not performed if histopathology reports from another center were compatible with pemphigus and BP and the diagnosis confirmed by ELISA. The clinical features of patients, DIF findings, anti-Dsg1, anti-Dsg3, and anti-BP180 autoantibody titers were recorded by ELISA. Sera from all patients and controls were stored at -20°C and then tested by the BIOCHIP mosaic-based IIF technique. This study was approved by the Ethical Committee of Akdeniz University, and informed consent was obtained from each of the contributors.

The BIOCHIP mosaic-based IIF technique

Anti-skin antibody detection by IIF was performed on the BIOCHIP Dermatology Mosaic 7 (EUROIMMUN, Lübeck, Germany) according to the manufacturer's instructions. This test is designed exclusively for the *in-vitro* determination of human antibodies in serum. In this approach, the substrates are no longer applied directly to microscope slides, but initially to thin glass slides. The millimeter-sized fragments, which are cut mechanically, are named BIOCHIPs. The BIOCHIPs were then glued onto microscope slides using automated assembly equipment. The miniature size of the BIOCHIPs means that the reaction fields of the slides can be supplemented with further BIOCHIP substrates if required (8) (this is referred to as the BIOCHIP Mosaic). Ten incubation fields exist on one standard-sized slide (each incubation field is for one of the patients), and each field is a mosaic of six different antigenic structures as a substrate. In this study, the substrates were: sections of primate monkey esophagus, primate (1 mol/L NaCl) salt-split skin (SSS) section, EU 90 cells transfected with the Dsg1, Dsg3, and C-terminal globular domain of BP230, and recombinant tetrameric BP180-NC16A-4X spots. The substrates were incubated with the sera of the patients and controls at a dilution of 1/10 in PBS-Tween at room temperature for 30 minutes. This constitutes the first incubation, allowing the antibodies to bind to the antigens on the BIOCHIP slide. After that, the slides were rinsed with a flush of PBS-Tween and immersed in PBS-Tween for 5 minutes. In the second incubation period, the attached antibodies were stained with fluorescein-labeled anti-human antibodies (FITC) in the dark at room temperature for a period of 30 minutes. Then, the slides were washed in the dark as described previously and evaluated visually by immunofluorescence microscopy. The sera of the patients and controls were blind-evaluated by the researcher on the same slide. The results were compared with the positive control sera of pemphigus and BP.

Diagnosis of pemphigus was established on the detection of intercellular staining (ICS) on the monkey esophagus and/or anti-Dsg1 and/or anti-Dsg3 autoantibodies using the BIOCHIP technique. Diagnosis of BP was established on the detection of the epidermal or epidermal and dermal deposition on SSS, and/or anti-BP180 and/or anti-BP230 autoantibodies also using the BIOCHIP technique.

ELISA test

For the detection of anti-Dsg1 and anti-Dsg3 antibodies, sera from the patients and control group were studied with a commercially available ELISA kit (EUROIMMUN, Lübeck, Germany) according to the manufacturers' instructions. Antigens coated on wells were an extracellular domain of Dsg1 (5 subdomains) and Dsg3 (5 subdomains) proteins produced

recombinantly in mammalian cells for anti-Dsg1 and anti-Dsg3 tests, respectively. For the detection of anti-BP180 antibodies, ELISA plates coated with immunogenic tetramer of NC16A domain (BP180) that are expressed in *Escherichia coli* were used (EUROIMMUN, Lübeck, Germany). For each test, three calibrators, containing the 2, 20, and 200 RU/mL analyte, as well as a positive and a negative control, were included in each run. Analyte concentrations in the samples of patients and controls were calculated in RU/mL using curves formed by calibrators. The concentrations below the 20 RU/mL were considered negative for the Dsg1, Dsg3, and BP180 tests. While concentrations between 20 and 200 RU/mL were quantified, any concentrations above 200 RU/mL were merely reported as being "above 200 RU/mL".

Statistical analyses

Statistical analyses were performed with SPSS (version 22.0). To detect the diagnostic value of the BIOCHIP technique, the results were compared with the results of the gold standard methods – the DIF and/or ELISA test – for validity; the sensitivity and the specificity values were calculated. Kappa values were calculated to measure the level of correlation (consistency) between the two methods. Kappa was obtained by the purification of the consistency due to chance from observed consistency:

Kappa = (observed consistency – consistency due to chance) / (1 – consistency due to chance)

Results were expressed as mean ± Standard Deviation. In all analyses, $P<0.05$ was considered statistically significant. While comparing the averages of the autoantibody titers obtained using the ELISA test, the Mann-Whitney U test was used, the non-parametric equivalent of the t-test, because of the high variance.

RESULTS

Data of patients with pemphigus

Twenty nine newly diagnosed and 16 relapsed patients with pemphigus and 35 individuals (17 women and 18 men) in the control group were included in the study. The demographic features of the patients and control group are shown in Table 1.

The mean duration after onset of disease of the newly diagnosed patients with pemphigus was 295.93±125.87 (mean ± Standard Deviation) days, while in patients with relapse it was 1360.37±169.46 days. Subtypes of pemphigus in the study group were pemphigus vulgaris (PV) (n=39), pemphigus foliaceus (PF) (n=3), pemphigus herpetiformis (PH) (n=1), IgA pemphigus (IgAP) (n=1) and pemphigus vegetans (PVE) (n=1). The mean age of the patients at onset of disease was 46.35±12.21 years. Mucosal involvements of the various sites were observed in 66.7%

Table 1. Demographic and clinical data of the patients in the pemphigus, bullous pemphigoid (BP), and control groups

Special Features	Pemphigus	BP	Control group
Sex			
Women	25	13	17
Men	20	5	18
Mean age ± Standard Deviation	51.22±12.76	60.87±23.55	42.42±16.02
Clinic			-
Newly diagnosed	29	16	
Relapsed	16	2	
Mean disease duration (days)			-
Newly diagnosed	295.93±125.87	196.5±169.46,	
Relapsed	1360.37±169.46	1382.5±479.33	
Number of blisters and/or erosions			-
<2	9	2	
3-5	5	1	
6-10	7	6	
>10	24	9	
Mucosal involvement (n)			-
Oral	36	3	
Nasal	6	0	
Conjunctival	3	0	
Genital	5	2	
Anal	2	0	

Table 2. The sensitivity and specificity of the BIOCHIP technique in the diagnosis of pemphigus

BIOCHIP results	DIF and/or ELISA results				Total	
	Positive	%	Negative	%	n	%
Positive	41	**91.1***	1	2.9	42	52.5
Negative	4	8.9	34	**97.1****	38	47.5
Total	45	100.0	35	100.0	80	100.0

**Sensitivity; **Specificity*

of the patients. Oral mucosa was the most common mucosal involvement, which was observed in 80.0% of the patients (Table 1).

Results of the BIOCHIP technique

The sera of 45 patients whose diagnoses were confirmed by DIF and/or ELISA were tested using the BIOCHIP technique. BIOCHIP was positive for pemphigus with at least one substrate in 41 of the patients. In the control group, BIOCHIP was positive in only 1 (2.9%) serum (false positivity). The sensitivity and specificity of BIOCHIP in the diagnosis of pemphigus was found to be 91.1% and 97.1%, respectively (Table 2).

Table 3. The correlation between the BIOCHIP technique and direct immunofluorescence (DIF) in detection of intracellular cytokine staining (ICS)

	DIF-ICS		
BIOCHIP-ICS	**Positive**	**Negative**	**Total**
Positive	13	11	24
Negative	6	7	13
Total	19	18	37

Correlation = 0.5405; Kappa = 0.076; *P*=0.642

A DIF examination was performed on 37 of the 45 patients, and 19 (51.4%) were positive. Since histopathological examination of 8 patients were reported as pemphigus, the biopsy for DIF examination was not taken again. DIF was not performed and the diagnosis was confirmed by ELISA in these patients. ICS staining on the monkey esophagus sections was observed in 31 (68.9%) patients with BIOCHIP. In

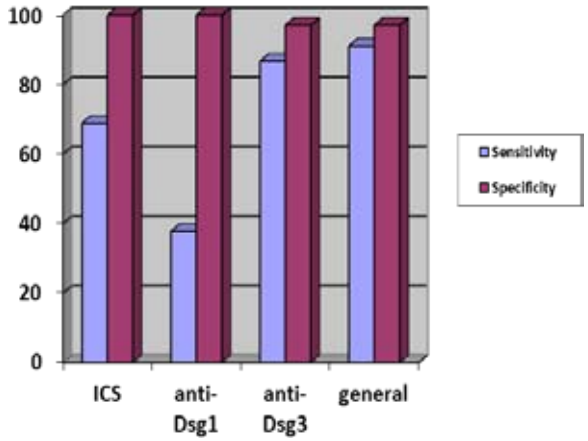

Figure 1. The sensitivity and specificity of the BIOCHIP technique in the diagnosis of pemphigus with intercellular staining (ICS), anti-Dsg1, and/or anti-Dsg3 positivity

detection of ICS staining, no statistically significant correlation was observed between BIOCHIP and DIF (Table 3). The specificity of ICS staining on esophagus section with BIOCHIP was 100% (Fig. 1).

ELISA was performed on the patients with pemphigus (n=45) and the control group (n=35): the anti-Dsg1 and anti-Dsg3 autoantibodies of the entire control group were found to be negative. The anti-Dsg1 autoantibodies in 25 patients and the anti-Dsg3 autoantibodies in 39 patients with pemphigus were positive in ELISA. BIOCHIP found anti-Dsg1 positivity in 17 patients, anti-Dsg3 in 39 patients with pemphigus. The sensitivity and specificity of BIOCHIP in detecting autoantibodies against Dsg1 in patients with pemphigus were 37.8% and 100%, respectively, and against Dsg3 were 86.7% and 97.1%, respectively (Fig-

Table 4. The correlation between the BIOCHIP technique and enzyme-linked immunosorbent assay (ELISA) in the detection of anti-Dsg1 autoantibodies

	BIOCHIP		
ELISA	**Positive**	**Negative**	**Total**
Positive	15	10	25
Negative	2	53	55
Total	17	63	80

Correlation = 0.8500; Kappa = 0.538; P<0.01

Table 5. The correlation between the BIOCHIP technique and enzyme-linked immunosorbent assay (ELISA) in the detection of anti-Dsg3 autoantibodies

	BIOCHIP		
ELISA	**Positive**	**Negative**	**Total**
Positive	37	2	39
Negative	3	38	41
Total	40	40	80

Correlation = 0.9375; Kappa = 0.659; *P*<0.01

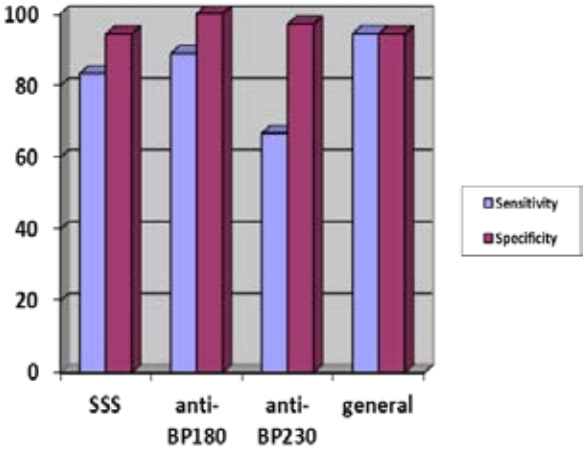

Figure 2. The sensitivity and specificity of the BIOCHIP technique in the diagnosis of BP with salt-split skin (SSS), anti-BP180 and/or anti-BP230 positivity

ure 1). The correlation between BIOCHIP and ELISA in the detection of anti-Dsg1 and Dsg3 was statistically significant (Table 4, Table 5).

In this study, the mean value of the anti-Dsg1 autoantibody titers obtained by ELISA was 132.12±72.79 RU/mL in the 17 patients with pemphigus whose anti-Dsg1 autoantibodies were positive in BIOCHIP. On the other hand, the mean value of the anti-Dsg1 autoantibody titers obtained by ELISA was 38.29±67.06 RU/mL in the 28 patients with pemphigus whose anti-Dsg1 autoantibodies were negative in BIOCHIP. The mean value of the anti-Dsg1 autoantibody titers detected by ELISA in the patients with pemphigus whose anti-Dsg1 autoantibody was positive in the BIOCHIP technique was significantly higher than in negative patients, and was statistically significant ($P<0.001$). Considering the similar relationship in anti-Dsg3 autoantibodies, the mean value of the anti-Dsg3 autoantibody titers obtained by ELISA was 166.74±55.91 RU/mL in the 39 patients with pemphigus whose anti-Dsg3 autoantibodies were positive in BIOCHIP. The mean anti-Dsg3 autoantibody titers obtained by ELISA were 66.67±103.28 RU/mL in the 6 patients with pemphigus whose anti-Dsg3 autoantibodies were negative in BIOCHIP. The mean value of the anti-Dsg3 autoantibody titers detected by ELISA in the patients with pemphigus whose anti-Dsg3 autoantibodies positive in BIOCHIP was higher than negative patients. However, this difference was not statistically significant ($P=0.063$).

Data of patients with BP

Sixteen newly diagnosed and 2 relapsed patients with BP, a total of 18, were included in the study. The demographic features of the patients and the control group are shown in Table 1. The mean duration after onset of disease for the newly diagnosed patients with BP was 196.5±169.46 days, while disease duration of the relapsed patients with BP was 1382.5±479.33 days. The mean age at onset of BP was found to be 56.89±24.07 years.

Results of the BIOCHIP technique

The sensitivity and specificity of BIOCHIP in the diagnosis of BP was found to be 94.4% and 94.3%, respectively (Table 6, Figure 2).

DIF was performed on 16 of the 18 patients with BP. It was positive as linear IgG/C3 deposition along the BMZ in 11 of the 16 patients. Since histopathological examinations of 2 patients were reported as BP, the diagnosis was confirmed by ELISA in these patients. BIOCHIP was found to be positive on SSS sections as epidermal side depositions for 15 of the 18 patients. In 2 sera in the control group, both dermal and epidermal side depositions were found by BIOCHIP on SSS sections. Anti-BP230 was also positive in one of these patients. Linear BMZ depositions were observed on the primate esophagus sections in 6 of the SSS-positive patients. The sensitivity and specificity of the SSS substrate of BIOCHIP for the diagnosis of BP was 83.3% and 94.3%, respectively.

ELISA was performed on 18 patients with BP and 35 controls. Anti-BP180 autoantibodies were positive in 15 of the patients with BP. These antibodies were negative in the control group. BIOCHIP was positive for purified antigen BP180 NC16A substrate in 16 of the patients. On the other hand, BIOCHIP was positive for the C-terminal globular domain of BP230 transfected EU 90 cells as substrate in 12 patients with BP.

Table 6. The sensitivity and specificity of the BIOCHIP technique in the diagnosis of bullous pemphigoid (BP)

BIOCHIP results	DIF and/or ELISA results				Total	
	Positive	%	Negative	%	n	%
Positive	17	**94.4***	2	5.7	19	35.8
Negative	1	5.6	33	**94.3****	34	64.2
Total	18	100.0	35	100.0	53	100.0

*Sensitivity; **Specificity; DIF: direct immunofluorescence; ELISA: enzyme-linked immunosorbent assay

Table 7. The correlation between the BIOCHIP technique and enzyme-linked immunosorbent assay (ELISA) in the detection of anti-BP180 autoantibodies

	BIOCHIP-anti-BP180		
ELISA-anti-BP180	**Positive**	**Negative**	**Total**
Positive	15	0	15
Negative	1	37	38
Total	16	37	53

Correlation = 0.9811; Kappa = 0.691; P<0.01

The sensitivity and specificity of BIOCHIP in detecting anti-BP180 autoantibodies was found to be 88.9% and 100%, respectively, and in detecting anti-BP230 autoantibodies it was 66.7% and 97.1%, respectively. The correlation between the BIOCHIP technique and ELISA in the detection of anti-BP180 autoantibodies was considered statistically significant (*P*<0.01) (Table 7).

DISCUSSION

We found that the BIOCHIP technique was highly sensitive and specific in the diagnosis of both pemphigus and BP. Other studies have investigated the sensitivity and specificity of the BIOCHIP technique in the diagnosis of pemphigus. Their results are shown in Table 8. Van Beek *et al.* studied BIOCHIP in the diagnosis of 65 patients with PV and 50 with PF and observed ICS positivity with sensitivity of 100% and 98%, respectively (9). On the other hand, ICS positivity with BIOCHIP was reported as 83% for PV in a study by Tampoia *et al.* (19). The specificity of ICS positivity by BIOCHIP in our study was found to be 100%. This rate was reported as between 89% to 96% in previous studies (9,10).

The sensitivity of detection of anti-Dsg3 autoantibodies (87%) was higher than anti-Dsg1 (38%) in this study (Figure 1). Van Beek *et al.* reported the BIOCHIP sensitivity for anti-Dsg1 in the diagnosis of PV and PF as 52% and 90%, respectively (9). Anti-Dsg1 antibodies diagnosed pemphigus by BIOCHIP with 100% specificity in this study. On the other hand, the BIOCHIP sensitivity and specificity of anti-Dsg3 antibodies in the diagnosis of PV was reported as 98.5% and 99.6%, respectively. Similarly, Tampoia *et al.* reported the sensitivity of anti-Dsg3 autoantibodies by BIOCHIP as 100%. But the sensitivity of anti-Dsg1 antibodies, in relation to their presence in a minority of the patients with PV, was reported as lower by BIOCHIP, at 33%. They reported the specificity of the anti-Dsg1 and anti-Dsg3 autoantibodies in the PV diagnosis as 98.5% and 100%, respectively. As a result they found that, in terms of detecting the anti-Dsg3 autoantibodies, BIOCHIP and ELISA methods displayed a strong correlation (10). Russo *et al.* also reported that BIOCHIP displayed high sensitivity in the diagnosis of PV (97.6%) and specificity (100%) in detecting anti-Dsg3 autoantibodies (11) (Table 8).

In our study, the mean titer of anti-Dsg1 detected by ELISA in patients with pemphigus whose anti-Dsg1 was positive in BIOCHIP was higher than BIOCHIP-negative patients; this was found to be statistically significant (*P*<0.001). Although a similar relationship was observed between anti-Dsg3-ELISA and anti-Dsg3-BIOCHIP, it was not statistically significant (*P*=0.063).

We found that the sensitivity of SSS substrate in the diagnosis of BP by BIOCHIP was 83.3%. Damoiseaux *et al.* reported SSS sensitivity with 1/10 and/or 1/100 dilution as 95%. The significant portion of the positivity was in the 1/100 dilution (7). However, since

Table 8. Studies on the sensitivity and specificity of the BIOCHIP technique in the diagnosis of pemphigus

Studies	Patients (n)	ICS		Anti-Dsg1		Anti-Dsg3	
		Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
van Beek *et al.* (9)	PV (n: 65) PF (n: 50)	%100.0 %98.0	%89.1 %89.1	%52.3 %90.0	%100.0 %100.0	%98.5 -	%96.6 %96.6
Tampoia *et al.* (10)	PV (n: 36)	%83.3	%95.5	%33.3	%98.5	%100.0	%100.0
Russo *et al.* (11)	PV (n: 42)	-	-	%19.0	%100.0	%97.6	%100.0
Current study	Pemphigus (n: 45) PV (n: 39) PF (n: 3) PVE (n: 1) IgA P (n: 1) PH (n: 1)	%68.9	%100.0	%37.8	%100.0	%86.7	%97.1

PV: pemphigus vulgaris; PF: pemphigus foliaceus; PH: pemphigus herpetiformis; IgAP: IgA pemphigus; PVE: pemphigus vegetans

Table 9. Studies on the the sensitivity and specificity of the BIOCHIP technique in the diagnosis of bullous pemphigoid (BP)

Studies	Patients	BMZ reactivity *(substrate)*		Anti-BP180		Anti-BP230	
		Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Damoiseaux *et al.* (7) (1:10 and/or 1:100 dilution)	BP n: 60	%95.0 *(esophagus/SSS)*	-	%88.3	%96.5	%38.3	%98.3
van Beek *et al.* (9) (1:10-1:3200 dilution)	BP n: 42	%98.8 *(esophagus and/ or SSS)*	%98.8	%100.0	%98.2	%54.8	%100.0
Tampoia *et al.* (10) (1:10 dilution)	BP n: 40	%50.0 *(esophagus)*	%100.0 *(esophagus BMZ)*	%90.0	%100.0	%30.0	%100.0
Zarian *et al.* (8)	BP n: 18	-	-	%83.3	%100.0	%39.0	%100.0
Current study (1:10 dilution)	BP n: 18	%83.3 *(esophagus and/ or SSS)*	%94.3	%88.9	%100.0	%66.7	%97.1

SSS: salt-split skin

Van Beek *et al.* conducted their study at 6 different dilutions, minimizing the prozone effect as a consequence, the sensitivity of BMZ reactivity was found to be higher (98.8%) than in the previous studies and the current study (Table 9).

BIOCHIP is more sensitive in the diagnosis of BP via the detecting of anti-BP180 than anti-BP230 autoantibodies and SSS positivity in most of the previous reports (7-10). In these studies, the specificities of SSS section, BP180 NC16A, and the C-terminal globular domain of BP230 transfected cells as substrates in BIOCHIP technique was over 95% in the diagnosis of BP (Table 9). Haik *et al.* reported that anti-BP180 autoantibodies were undetectable by BIOCHIP in 3 patients whose anti-BP180-ELISA titers were low (8). Similarly, in the current study, anti-BP180 autoantibodies were undetectable by BIOCHIP in 2 patients whose ELISA was negative.

CONCLUSION

This study has shown that the BIOCHIP technique is a valuable screening test in the diagnosis of both pemphigus and BP with a high degree of sensitivity and specificity in the Turkish population with different genetic backgrounds. It appears that the most sensitive substrates for the diagnosis of pemphigus and BP were transfected cells with Dsg3 and BP180 NC16A, respectively. BIOCHIP may serve as a less time-consuming screening test in the differential diagnosis of ABD. However, the relatively low number of samples and testing with only one dilution are the main limitations of the study. Another limitation is that DIF was not performed in all patients, and the low rate of DIF positivity also can be a bias in comparison with BIOCHIP. Although BIOCHIP seems to be highly sensitive and specific for pemphigus and BP, the value of this innovative technique should also be evaluated for the diagnosis of other ABD.

Acknowledgements

This work was supported by The Scientific Research Projects Coordination Unit of Akdeniz University. Project Number: 2014.04.0103.008.

References:

1. Uzun S, Durdu M, Akman A, Gunasti S, Uslular C, Memisoglu HR, *et al.* Pemphigus in the Mediterranean region of Turkey: a study of 148 cases. Int J Dermatol 2006;45: 523-8.
2. Di Zenzo G, Della Torre R, Zambruno G, Borradori L. Bullous pemphigoid: from the clinic to the bench. Clin Dermatol 2012;30:3-16.
3. Joly P, Litrowski N. Pemphigus group (vulgaris, vegetans, foliaceus, herpetiformis, brasiliensis). Clin Dermatol 2011;29:432-6.
4. Kershenovich R, Hodak E, Mimouni D. Diagnosis and classification of pemphigus and bullous pemphigoid. Autoimmun Rev 2014;13:477-81.
5. Schmidt E, Zillikens D. Modern diagnosis of autoimmune blistering skin diseases. Autoimmun Rev 2010;10:84-9.
6. Schmidt E, Dähnrich C, Rosemann A, Probst C, Komorowski L, Saschenbrecker S, *et al.* Novel ELISA systems for antibodies to desmoglein 1 and 3: correlation of disease activity with serum autoantibody levels in individual pemphigus patients. Exp Dermatol 2010;19:458-63.

7. Damoiseaux J, van Rijsingen M, Warnemünde N, Dähnrich C, Fechner K, Tervaert JW. Autoantibody detection in bullous pemphigoid: clinical evaluation of the EUROPLUS™ Dermatology Mosaic. J Immunol Methods 2012;382:76-80.
8. Zarian H, Saponeri A, Michelotto A, Zattra E, Belloni-Fortina A, Alaibac M. BIOCHIP technology for the serological diagnosis of bullous pemphigoid. ISRN Dermatol 2012;2012:237802.
9. van Beek N, Rentzsch K, Probst C, Komorowski L, Kasperkiewicz M, Fechner K, *et al.* Serological diagnosis of autoimmune bullous skin diseases: prospective comparison of the BIOCHIP mosaic-based indirect immunofluorescence technique with the conventional multi-step single test strategy. Orphanet J Rare Dis 2012;7:49.
10. Tampoia M, Zucano A, Villalta D, Antico A, Bizzaro N. Anti-skin specific autoantibodies detected by a new immunofluorescence multiplex BIOCHIP method in patients with autoimmune bullous diseases. Dermatology 2012;225:37-44.
11. Russo I, Saponeri A, Peserico A, Alaibac M. The use of BIOCHIP immunofluorescence microscopy for the diagnosis of Pemphigus vulgaris. Acta Histochem 2014;116:713-6.