Original Article

Comparative Analysis of BIOCHIP Mosaic-based Indirect Immunofluorescence with Direct Immunofluorescence in Diagnosis of Autoimmune Bullous Diseases: A Cross-Sectional Study

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Abstract

Background: Autoimmune bullous diseases (AIBD) are a heterogeneous group of diseases characterized by autoantibodies against desmosomal proteins in the pemphigus group of disorders and adhesion molecules of the dermal-epidermal junction in pemphigoid group of diseases. Direct immunofluorescence (DIF) establishes the diagnosis of AIBD by demonstrating intercellular deposits of IgG and C3 in case of pemphigus and linear deposits of IgG and C3 along the basement membrane zone (BMZ) in bullous pemphigoid (BP). BIOCHIP mosaic-based indirect immunofluorescence (IIF), a novel diagnostic approach employs detection of characteristic staining pattern and target antigens in a single miniature incubation field. **Aim:** To compare the BIOCHIP mosaic-based IIF with DIF in the diagnosis of AIBD. **Materials and Methods:** A total of 40 patients of AIBD in the active phase of the disease were included in the study. Skin biopsy was done in these patients for DIF study and serum was subjected to BIOCHIP mosaic-based IIF assay. The results were then compared. **Results:** DIF revealed a diagnosis of Pemphigus in 18 patients and BP in 22 patients. BIOCHIP showed a diagnosis of pemphigus in 18 patients, BP in 18 patients and floor pattern staining in four patients, which could be attributed to any of the floor pattern staining subepidermal blistering disease. **Limitations:** Small sample size, lack of control group and no comparison made with ELISA. **Conclusion:** This study concludes that the result of BIOCHIP shows correlation with the DIF and can be used as a first line-screening tool in the diagnosis of AIBD.

Keywords: *Autoimmune bullous skin diseases, BIOCHIP mosaic, direct immunofluorescence, indirect immunofluorescence*

Introduction

Autoimmune bullous diseases (AIBD) include diverse group of skin diseases characterized by autoantibodies against desmosomal proteins in case of pemphigus group of diseases and components of basement membrane zone (BMZ) in pemphigoid diseases. Diagnosis of AIBD is based on the combination of characteristic clinical features, histopathological findings, direct immunofluorescence (DIF), indirect immunofluorescence (IIF) or enzyme-linked immunosorbent assay (ELISA) for target antigens. BIOCHIP mosaic, a novel diagnostic technique employs detection of target antigens and characteristic-staining pattern, in a single miniature incubation field.[1]

Materials and Methods

The present study was a cross-sectional study conducted during September 2018-July 2019 in the department of dermatology and pathology in a tertiary care hospital. Institutional ethics committee approval was obtained prior to the commencement of the study. A total of 40 patients with suspected AIBD were included in the study. AIBD patients under treatment and clinical remission were excluded from the study. All the participants were informed regarding the study and samples were collected after written consent. Skin biopsy was obtained from uninvolved perilesional skin for DIF and 5 ml of blood was obtained for BIOCHIP mosaic evaluation. Subsequently, the results of DIF and BIOCHIP mosaic were compared.

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BIOCHIP mosaic

Procedure

The Dermatology mosaic 7 BIOCHIP (Euroimmun, Germany) was used in this study. The incubation field in BIOCHIP slide has mosaic of six different substrates: primate oesophagus, primate salt split skin, transfected cells with desmoglein 1 (Dsg1), desmoglein 3 (Dsg3), C-terminal globular domain of the bullous pemphigoid antigen 230 (BP230) and recombinant antigenic dots of tetrameric bullous pemphigoid antigen 180–non-collagenous 16 A domain (BP180-NC16A). For BIOCHIP mosaic, 5 ml of blood is taken by venipuncture in a plain test tube. The blood sample is then centrifuged at the rate of 3000 rotations per minute for 10 minutes for serum separation. 50µL of serum is mixed with 450 µL of buffer [1:10 dilution]. 30 µL of the above mixture is incubated in substrate wells for 30 minutes and washed with a buffer for 5 minutes. After that, fluorescent conjugate 25 µL is added and incubated again for 30 minutes and washed with a buffer for 5 minutes. The slide is then mounted for interpretation under a fluorescent microscope. All the tests were run with the positive controls provided with the kit.

Interpretation

Primate oesophagus shows fine granular fluorescence of intercellular space (ICS) staining pattern in case of pemphigus and linear basement membrane zone (BMZ) staining pattern in pemphigoid diseases. In salt split skin substrate there will be a linear fluorescence pattern in the roof of the split in bullous pemphigoid and floor of the split in epidermolysis bullosa acquisita and rare variants like anti-laminin 332 pemphigoid and anti-P200 pemphigoid. Dsg 1, Dsg 3 and BP 230 transfected cells show fine granular cytoplasmic fluorescence and BP 180 substrate shows diamond-shaped fluorescence in positive cases.

In the case of DIF, an ICS pattern with IgG and/or C3 is diagnostic of pemphigus. A linear BMZ-staining pattern with predominantly IgG and/or C3 points to the diagnosis of pemphigoid. Standards for reporting of diagnostic accuracy for DIF included type of antibody (IgG, IgA or IgM), intensity (1 + to 3+) and pattern of staining (ICS or BMZ pattern). For BIOCHIP reporting was done as per the interpretation described above.

Results

During the study period, 40 patients fulfilled the inclusion criteria, which included 16 males and 24 females. The age of these patients ranged between 19 and 80 years with a mean age of 53.8 years. Among the study participants, a clinical diagnosis of pemphigus was entertained in 18 patients (16 pemphigus vulgaris and 2 pemphigus foliaceus) and bullous pemphigoid in 22 patients based on characteristic clinical findings.

In pemphigus group, DIF showed characteristic ICS pattern with IgG and C3 in 9 patients and IgG alone in 9 patients. In the pemphigoid group, DIF showed a linear BMZ pattern with IgG and C3 in 14 patients, IgG alone in 4 patients and C3 alone in 4 patients [Figure 1a and b].

BIOCHIP mosaic was then probed with sera from these patients. In the pemphigus group, primate oesophagus showed ICS pattern in all 18 patients, Dsg 1 and 3 were positive in 13 patients, Dsg 3 was positive in 3 patients and Dsg 1 was positive in 2 patients. In general, Dsg 1 was positive in 15 patients and Dsg 3 was positive in 16 patients [Figure 2a and b]. In pemphigoid group, primate oesophagus showed linear BMZ pattern in all 22 patients, BP 180 and BP 230 were positive in 14 patients, BP 180 in 3 patients, and BP 230 in 1 patient. Overall BP 180 was positive in 17 patients and BP 230 in 15 patients [Figure 3a and b]. Salt split skin substrate showed a roof pattern in 18 patients and floor pattern in 4 patients [Figure 4a, b and Tables 1, 2].

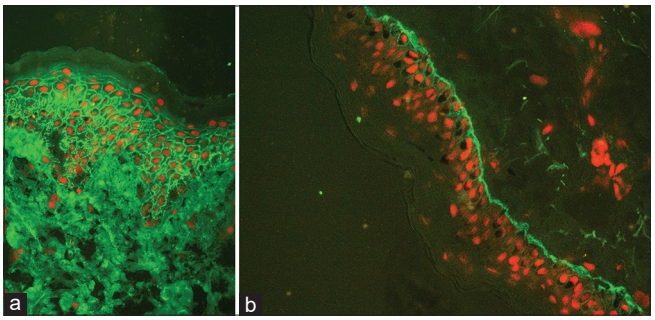

Figure 1: (a) DIF – IgG Intercellular staining pattern in the pemphigus vulgaris (× 200). (b) DIF–IgG linear Basement membrane zone-staining pattern in bullous pemphigoid (×200)

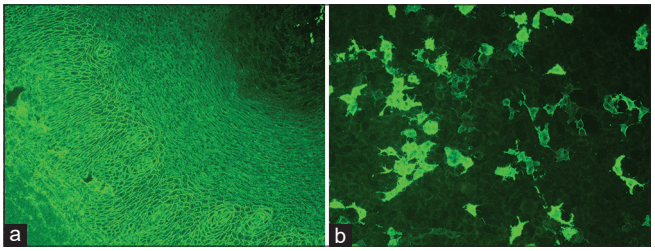

Figure 2: (a) Primate Oesophagus – Intercellular staining pattern (×200). (b) Positive fluorescence in Dsg 3 Transfected cells (×200) (similar fluorescence will be seen in Dsg 1, BP 230 transfected cells)

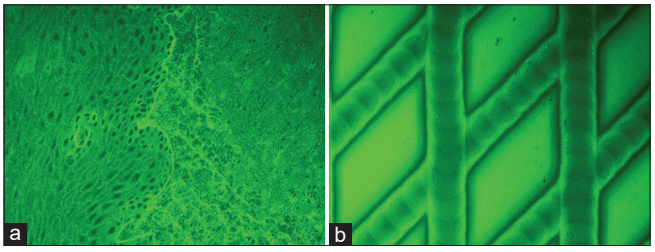

Figure 3: (a) Primate Oesophagus – Basement membrane zone-staining pattern (×200). (b) Positive fluorescence in BP 180 Tetrameric dots (×200)

Table: 1 Results of BIOCHIP mosaic in the pemphigus group (*n*=18)

Substrate	Result	Number of patients	Percentage
Primate oesophagus	Intercellular staining pattern	18	100
Dsg 1	Positive	15	83.3
Dsg 3	Positive	16	88.8

Dsg – Desmoglein

Table: 2 Results of BIOCHIP mosaic in pemphigoid group (*n*=22)

Substrate	Result	Number of patients	Percentage
Primate oesophagus	Linear basement membrane zone pattern	22	100
Salt split skin	Roof staining	18	81.8
Salt split skin	Floor staining	04	18.2
BP 180	Positive	17	77.3
BP 230	Positive	15	68.2

BP – Bullous pemphigoid antigen

BIOCHIP mosaic results showed a concordance of 100% in the diagnosis of pemphigus with DIF. In case of the pemphigoid group in comparison with DIF, BIOCHIP showed the diagnosis of bullous pemphigoid in 18 patients. The remaining four patients showed a floor pattern in salt split skin substrate and was negative for any of the target antigens.

The correlation between BIOCHIP mosaic and DIF in the diagnosis of AIBD was assessed using Spearman's correlation coefficient and it was found to be statistically significant.

Discussion

The diagnosis of AIBD involves a multistep approach-combining clinical feature, histopathology, DIF, IIF or ELISA. However, the gold standard remains the visualisation of autoantibodies in the skin or mucosa by DIF of perilesional skin biopsy.[1] BIOCHIP mosaic-based IIF is a new diagnostic technique that combines a simultaneous assessment of the staining pattern and identification of target antigens in a single field.[2] Studies assessing the validity of biochip have been conducted in Germany, Italy, Turkey, Poland and Australia. There is a need to assess the diagnostic value of the BIOCHIP in population groups of various ethnicities.[2]

Van Beek *et al*. from Germany were the first to evaluate the utility of BIOCHIP mosaic in the diagnosis of AIBD by comparing it with the multistep algorithm described by Schimdt and Zillikens.[1,3] The results of their study concluded that the diagnostic efficacy of the BIOCHIP mosaic was comparable with the conventional multistep procedure in the diagnosis of AIBD.[1]

Tampoia *etal.* investigated the value of the BIOCHIP method by comparing it with two commercially available

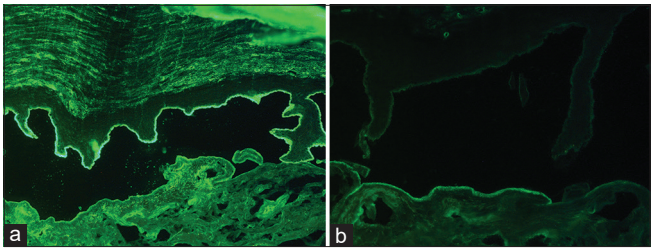

Figure 4: (a) Salt split skin – Roof pattern (×200). (b) Salt split skin – Floor pattern (×200)

ELISA tests (MBL, Japan and EuroImmun, Germany) in the diagnosis of pemphigus and pemphigoid.[4] Later Russo *et al.*from Italy and Özkesici*et al.*from Turkey evaluated the utility of BIOCHIP in the diagnosis of AIBD by comparing it with ELISA.[5,6] These studies concluded that the BIOCHIP method has a diagnostic accuracy comparable to ELISA.[4-6] However in the present study, no such comparison was made with ELISA.

Prussmann and co-workers from Germany studied the prevalence of pemphigus autoantibodies in the general population with a total of 7063 participants using the BIOCHIP method. Their study revealed a very low prevalence of autoantibodies in a large cohort of healthy individuals. Also functional analysis revealed differences between pathogenic autoantibodies in diseased individuals and antibodies detected from healthy donors.[7] The present study did not include any healthy controls and samples were collected only from cases of suspected AIBD.

Russo *etal.* in their study used the serum and salivary samples to detect anti-Dsg autoantibodies for diagnosis of pemphigus and concluded that saliva is not a suitable sample for BIOCHIP.[8] Gornowicz-Porowska *etal.* from Poland compared the original BIOCHIP method with modified BIOCHIP method using monoclonal IgG instead of routine IgG and concluded that modified BIOCHIP has as higher sensitivity and specificity.[9] The present study utilized routine commercial IgG provided with the kit.

Similar to the previous studies, the current study also revealed that Dsg 3 and BP 180 were the commonly detected antigens using BIOCHIP in pemphigus and pemphigoid groups, respectively [Table 3].[1,4-6,8-12,13]

The higher correlation in pemphigus group could be attributed to the fact that in pemphigus diseases only two main target antigens are there, both of which are present in BIOCHIP substrates; whereas in the pemphigoid group apart from BP 180 and 230, other antigens in the basement membrane zone may be the target, which needs evaluation with immunoblotting.

In the present study, out of 22 patients in the pemphigoid group, four patients showed a floor pattern of staining in salt split skin.These four patients revealed a linear BMZ pattern in primate oesophagus and were negative for any of the target antigens. These cases could be attributed to

Table 3: Percentage of positivity of target antigens in the diagnosis of AIBD by BIOCHIP mosaic in previous studies

Authors	Country	Year	Pemphigus (*n*)	Bullous Pemphigoid (*n*)	Dsg 1 (%)	Dsg 3 (%)	BP180 (%)	BP230 (%)
Van Beek *et al*.[1]	Germany	2012	65	42	52.3	98.5	100	54.8
Tampoia *et al*.[4]	Italy	2012	36	40	33.3	100	90	40
Damoiseaux *et al*.[11]	Netherlands	2012	-	60	-	-	88	38
Zarian *et al*.[12]	Italy	2012	-	18	-	-	83.3	39
Russo *et al*.[5]	Italy	2014	42	-	19	97.62	-	-
Özkesici *et al*.[6]	Turkey	2017	45	18	37.8	86.7	88.9	66.7
Russo *et al*.[8]	Italy	2017	8	-	12.5	100		
Gornowicz-Porowska *et al*.[9]	Poland	2017	19	37	68.4	63.2	89.2	24.3
Yang *et al*.[10]	Australia	2019	23	28	75	67.5	55.3	65.8
Tirumalae *et al*.[13]	India	2019	37	29	73	86	95	14
Current study	India	2019	18	22	83.3	88.8	77.3	68.3

AIBD=Autoimmune bullous disease, Dsg=Desmoglein, BP=Bullous pemphigoid antigen.

floor pattern-staining diseases like epidermolysis bullosa acquisita, anti-laminin 332 pemphigoid or anti-P-200 pemphigoid, which needs further evaluation with immunoblotting.[14-16] This finding of floor pattern in 4 out of 22 cases of pemphigoid group using BIOCHIP is a significant observation. In an Indian study by Tirumalae *et al.*, similar findings were noted in four cases that showed either roof or floor positivity on salt split skin substrate with negative results in other substrates. They categorized these cases as unclassified subepidermal diseases.[13]

The advantage of BIOCHIP is that the combination of different substrates in the same field allows for concurrent evaluation of characteristic-staining pattern, identification of target antigens at once. Also, it facilitates distinction among the various types of AIBD. Further, this multiparametric technique is cost- and time-effective compared to the conventional multi-step approach.[17] However, it has limitations in categorisation of pemphigoid diseases due to restricted antibody coating. This could be overcome by testing additional target antigens or immunoblotting.

The literature search revealed only a few studies investigating the validity of the BIOCHIP IIF in the diagnosis of AIBD. These studies had concluded that the BIOCHIP method has a high degree of sensitivity and specificity in the diagnosis of AIBD.[2] The results of the present study show that the diagnosis of AIBD by BIOCHIP is showing a statistically significant correlation with that of DIF.

Limitations of this study include small sample size, lack of control group and no comparison made with ELISA.

Conclusion

BIOCHIP mosaic shows a good correlation with DIF. BIOCHIP mosaic is a non-invasive, rapid diagnostic technique that can detect the characteristic-staining pattern and target antigens in a single miniature incubation field and can be used as firstline tool in the diagnosis of AIBD.

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Conflicts of interest

There are no conflicts of interest.

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