Evaluation of the Role of BIOCHIP Mosaic Based Indirect Immunofluorescence and ELISA BP 180 and BP 230 Autoantibodies in the Diagnosis of Bullous Pemphigoid Patients

Abstract

**Background:** Bullous pemphigoid (BP) is characterized by tissue-bound and circulating Immunoglobulin G (IgG) autoantibodies against BP 180 and BP 230. Diagnosis of BP is a multi-step procedure. Enzyme-linked immunosorbent assay (ELISA) is a quantitative analysis of target antigens, whereas BIOCHIP mosaic-based indirect immunofluorescence (IIF) has a combination of screening and target antigen-specific substrates in a single miniature incubation field. This study is done to compare BIOCHIP mosaic based IIF and ELISA for the diagnosis of BP.

**Materials and Methods:** A total of 42 biopsy and/or direct immunofluorescence (DIF) proven BP patients were included in the study. Serum was subjected to BIOCHIP mosaic-based IIF and ELISA. The results were then compared. **Results:** Using ELISA, the sensitivity of BP 180 and BP 230 was 92.3% and 54.5%, respectively. The sensitivity of BP 180 and BP 230 by BIOCHIP was 77.4% and 60%, respectively. The association between ELISA and BIOCHIP was analyzed using the Chi-square test and was found to be statistically significant with a P value ≤ 0.05. **Conclusion:** Our study concluded that both BIOCHIP and ELISA showed comparable sensitivity in diagnosing BP. Both are non-invasive, less time-consuming, and provide fast results. However, BIOCHIP can delineate bullous pemphigoid from other sub-epidermal bullous diseases.

**Keywords:** BIOCHIP mosaic, bullous pemphigoid, ELISA

Introduction

Bullous pemphigoid (BP) is a common sub-epidermal immunobullous disorder characterized by tissue-bound and circulating Immunoglobulin G (IgG) autoantibodies against two components of hemidesmosomes, BP 180 (BP Ag2) and BP 230 (BP Ag1).[1] The diagnosis of BP is a multi-step procedure comprising histopathology, direct immunofluorescence (DIF), indirect immunofluorescence (IIF), enzyme-linked immunosorbent assay (ELISA), and BIOCHIP mosaic-based IIF. ELISA is a quantitative analysis of target antigens, whereas BIOCHIP mosaic-based IIF is a semi-quantitative analysis that has a combination of screening and target antigen-specific substrates in a single miniature incubation field.[2] This study is done to compare BIOCHIP mosaic-based IIF and ELISA BP 180 and BP 230 autoantibodies in the diagnosis of BP.

Materials and Methods

The present study was a cross-sectional study conducted from January 2020 to May 2021 in the department of dermatology, pathology, and microbiology in a tertiary care hospital. Institutional ethics committee approval was obtained prior to the commencement of the study. A total of 42 biopsy and/or DIF-proven BP patients were included in the study. Patients in clinical remission and other bullous disorders were excluded. All patients were informed regarding the study, and samples were collected after written consent. 5ml of blood was withdrawn from each patient. Serum was subjected to BIOCHIP mosaic-based IIF assay[3] and ELISA. Euroimmun ELISA kit was used in this study with a cut-off value of 20 RU/ml. The Dermatology mosaic 7 BIOCHIP (Euroimmun, Germany) was used in this study, in which BP 230 transfected cells show fine...
granular cytoplasmic fluorescence [Figure 1a] and BP 180 substrate show diamond shaped fluorescence [Figure 1b]. The results of ELISA and BIOCHIP were compared and analyzed.

**Results**

Forty-two patients were included in the study, out of which there were 22 males and 20 females. Auto antibodies to BP 180 by ELISA was positive in 73.8% of patients, and BP 230 was positive in 23.8% of patients. Both BP 180 and BP 230 were positive in 19% of patients.

By BIOCHIP, BP 180 was positive in 61.9% of patients, BP 230 was positive in 26.1% of patients, and 7.2% showed both BP 180 and BP 230 positivity.

The sensitivity of BP 180 by ELISA in our study was found to be 92.3%, and the sensitivity of BP 230 by ELISA was found to be 54.5%. The sensitivity of BP 180 by BIOCHIP was found to be 77.4%, and the sensitivity of BP 230 by BIOCHIP was 60%. The correlation between BIOCHIP mosaic and ELISA BP 180 and BP 230 autoantibodies in the diagnosis of BP was assessed using the Chi-square test and was found to be statistically significant with a P value ≤ 0.05.[Tables 1 and 2].

**Discussion**

BP, an autoimmune subepidermal bullous skin disease, is characterized by cutaneous and/or mucosal blistering lesions. It is diagnosed histopathologically by subepidermal split and the presence of immunoglobulins and complement at the basement membrane zone (BMZ) by DIF. Serologically, ELISA and BIOCHIP mosaic diagnostic techniques detect target antigens, in addition BIOCHIP also detects staining patterns. The diagnosis of BP 180 by ELISA in various studies described high sensitivity and specificity (53–100% and 94–100%, respectively).[4-6] However, anti-BP 230 autoantibodies were detected by ELISA in 57–63% of BP cases.[5,7-9] Our study also showed high sensitivity in detecting both BP 180 and BP 230 by ELISA.

So far, various studies have been done to evaluate the serological diagnosis in bullous pemphigoid by the BIOCHIP method. The BIOCHIP has demonstrated itself with high sensitivity for BP 180 (83.3–100%) but poor sensitivity for BP 230 (24.3–66.7%).[10-13]

Zarian et al.[14] from Italy demonstrated that 83.33% of BP patients were positive for BP 180 autoantibodies while autoantibodies against BP 230 were detected only in 39% of BP patients. This study concluded that BIOCHIP is an alternative to indirect immunofluorescence and ELISA.

Ozkesici and his colleagues from Turkey compared BIOCHIP with DIF and ELISA in patients with BP. Sensitivity in diagnosing BP using BIOCHIP and ELISA was 94.4% and 94.3%, respectively. There was a statistically significant correlation between BIOCHIP and ELISA in the detection of BP 180 antibodies.[15] Our study also demonstrated sensitivity in detecting BP 180 and BP 230 by BIOCHIP and also showed a statistically significant correlation with that of ELISA.

Although both ELISA and BIOCHIP showed sensitivity in diagnosing BP, the main advantage of the BIOCHIP is the simultaneous, multiparametric analysis of all relevant antibodies against target antigens and also to identify characteristic staining patterns so that BIOCHIP can delineate BP from other sub-epidermal bullous diseases, like epidermolysis bullosa acquisita, anti-laminin 332 pemphigoid, and anti-P-200 pemphigoid.[3]

Limitations of this study include small sample size and the lack of a control group. We performed a biopsy and/or DIF on a few patients, but both were done on majority of the patients.

### Table 1: The correlation between the BIOCHIP technique and ELISA in the detection of anti-BP 180 autoantibodies

| ELISA Anti-BP 180 | BIOCHIP Anti-BP 180 |
|-------------------|---------------------|
| Positive          | 26                  |
|                   | 7                   |
| Total             | 33                  |

The association of BP 180 between ELISA and BIOCHIP was analyzed using the Chi-square test and found to be statistically significant with a P value ≤0.05

### Table 2: The correlation between the BIOCHIP technique and ELISA in the detection of anti-BP 230 autoantibodies

| ELISA Anti-BP 230 | BIOCHIP Anti-BP 230 |
|-------------------|---------------------|
| Positive          | 6                   |
|                   | 4                   |
| Total             | 10                  |

The association of BP 230 between ELISA and BIOCHIP was analyzed using the Chi-square test and found to be statistically significant with a P value ≤0.05
Conclusion

Our study concluded that both BIOCHIP and ELISA showed comparable sensitivity in diagnosing bullous pemphigoid. Both tests are non-invasive, less time-consuming, and provide fast results. However, BIOCHIP can delineate bullous pemphigoid from other subepidermal bullous diseases.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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