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Original Article

Role of Direct Immunofluorescence in the Diagnosis of Immunobullous Disorders

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ABSTRACT

Introduction: Immunobullous disorders of skin are characterized by formation of autoantibodies against tissue proteins which lead to blistering of skin and mucous membrane. The most common diagnostic technique for the investigation of immunobullous disorders include conventional histopathology. Other tests that are essential as confirmative tests are Direct and Indirect immunofluorescence. In general, Clinical features and histological confirmation can make a correct diagnosis of immunobullous disorders. Confirmatory studies such as immunofluorescence will help in diagnosis of cases where histopathological features alone are not diagnostic.

Materials and Methods: In the present study 35 cases of immunobullous disorders, diagnosed based on clinical features, Tzanck smear and Histopathology were subjected to Direct Immunofluorescence (DIF) and the results were analysed.

Observations: Of these 35 cases, pemphigus vulgaris was found to be the most common disorder (43%) followed by bullous pemphigoid (34%). This was followed by CBDC (5%). Pemphigus foliaceus, IgA pemphigus, dermatitis herpetiformis, linear IgA dermatoses, bullous SLE, Lichen Planus Pemphigoides constituted 3% respectively. Overall, DIF was found to be positive in 97.1% of the cases. DIF was positive in 100% of the cases in Pemphigus group and in 94.4% of subepidermal disorders. It was positive in all cases of Pemphigus vulgaris and Bullous pemphigoid. DIF was also compared with Histopathological diagnosis which was specific in 85.7% of cases.

Conclusion: The present study reaffirms that apart from new sophisticated tests (immunoblotting, immunoprecipitation, immunoelectron microscopy), the diagnosis of Immunobullous disorders still relies on DIF findings in most laboratories. However, it is not a substitute to clinical or histopathological diagnosis but rather complementary to it.

Keywords: *Immunobullous Disorders, Direct Immunofluorescence, Histopathology.*

INTRODUCTION

Blistering diseases are a group of dermatological disorders which are common all over the world. Although blisters have drawn great attention of medical caregivers throughout history, only modern times have seen the origin of a clear classification of these diseases. The blisters (vesicles, bullae) occur as a primary disease manifestation or as secondary phenomenon. Most common conditions that result in blisters can be classified broadly into genetic abnormality, physical, inflammatory, immunological reactions and drug reaction. Of these, immunological reaction is the prominent cause. Immuno-bullous disorders of skin are characterized by formation of autoantibodies against tissue proteins which lead to blistering of skin and mucous membrane. Recent advances in investigative dermatology have made great advances in the understanding of clinical behaviour and molecular nature of autoimmune bullous disorders. The most important techniques for the investigation of immunobullous disorders include conventional histopathology, confirmative tests like direct and indirect immunofluorescence.(1) Though the newer sophisticated tests like immunoblotting,

immunoprecipitation, immunoelectron microscopy may redefine the diagnosis of autoimmune bullous disorders, they do not replace the conventional methods [2,3] like Clinical features and histopathological diagnosis. Confirmatory studies such as immunofluorescence will help in diagnosis of cases where histopathological features alone are uncertain. Corticosteroids remain the main stay of treatment which has played a great role in reducing the mortality due to immuno-bullous disorders. Treatment has been improvised by the combinations of steroids with various other immunosuppressives. This study is undertaken to evaluate the role of direct immunofluorescence in the diagnosis of immunobullous disorders.

MATERIALS AND METHODS

This study was conducted in a tertiary care centre, Chennai over a period of One year. The type of study was cross sectional observation study. All patients suspected to have immunobullous disorders were included in the study. In the study period, 35 cases of immunobullous disorders were diagnosed based on clinical features, Tzanck smear and Histopathology and was subjected to Direct Immunofluorescence (DIF) and the role of DIF evaluated in all the patients.

STUDY PROCEDURE

Patients with clinical features of immunobullous disorders were enrolled for the investigation. Nikolsky's sign was looked for and Tzanck smear was done in all the patients and smears were examined for acantholysis. Histopathological evaluation was done in all patients and the diagnosis was considered specific if it showed characteristic bulla with features of suspected disease, for example acantholytic cells in pemphigus. The diagnosis was considered to be non-specific if the biopsy showed neither bulla nor any features diagnostic of a particular group of blistering diseases. Based on above features a diagnosis of immunobullous disorder was made and the role of DIF was evaluated in all the patients. A 3-4mm punch biopsy was taken from the unblistered perilesional area under local anaesthesia. The specimen was transferred in saline and transported to pathology laboratory immediately. The specimen was then washed thrice in phosphate-buffered saline and was embedded in the optimal cutting temperature compound, Snap frozen and then 4-5 µm sections were cut (minimum 10 sections). Two sections were layered on each slide and the slides were then air dried and stained. Before staining, the sections were again washed in PBS (phosphate buffer solution) to remove unbound serum proteins and Optimally diluted FITC-labelled monospecific immunoglobulins IgG, IgA, and IgM, C3 were added and incubated at 37 °C for 45 min to 1 h. The sections were again washed in PBS thrice to remove unbound antibodies and mounted in a buffered glycerine at neutral pH and then examined under fluorescence microscope.

RESULTS

The results obtained were analysed as a whole, between two subgroups (pemphigus group and subepidermal disorders) and with regards to individual groups. Among all the immunobullous disorders, pemphigus vulgaris was found to be the most common disorder (43%) followed by bullous pemphigoid (34%). This was followed by Chronic bullous disease of Childhood (CBDC) which was 5%. Pemphigus foliaceus, IgA pemphigus, dermatitis herpetiformis (DH), linear IgA dermatoses (LAD), bullous SLE, and Lichen Planus pemphigoides (LPP) constituted 3% of cases respectively. The various distribution of immunobullous disorders is shown in Figure 1. In the present study, the youngest age group was 5 years old and the oldest age included 85 years. The maximum number of cases were in the age group of 31-40 years. In the present study there were totally 20 females and 15 males. Pemphigus group had female predominance while subepidermal disorders had male predominance. Overall DIF was found to be positive in 97.1% of the cases. The role of DIF was analysed and was compared with histopathalogy. In general, the site of immune deposits was in the intercellular space in 49% of cases, dermo-epidermal junction in 46%, and papillary tip in 3 % of cases. The pattern was fishnet in 49%, linear in 43%, and granular in 6% of the cases. Histopathology was specific in 85.7% of the cases.

Under pemphigus group, in the present study we had 15 cases of pemphigus Vulgaris, and one case of IgA pemphigus and pemphigus foliaceus. Out of them, Mucosal lesions were seen in 88%, Nikolsky was positive in 94%, and Tzanck showed acantholytic cells in 82%. In Pemphigus Vulgaris, mucosal involvement and Nikolsky's sign was positive in 100% of the cases and Tzanck was positive in 87% of the cases. DIF was positive in all (100%) of the cases. The most common immune deposits were of IgG type, followed by C3 and IgA. Pemphigus Vulgaris showed IgG and C3 deposits in all cases. Pemphigus foliaceus showed positivity for IgG alone and IgA Pemphigus for IgA alone. All disorders were negative for IgM. The deposits were intercellular in all the cases and showed fishnet pattern. Histopathology was positive in 87% of Pemphigus Vulgaris cases. In the present study histopathology was diagnostic in Pemphigus foliaceus and IgA Pemphigus. The clinical features, histopathology and DIF pictures are shown in Figures 2.

Among sub epidermal bullous disorders group, in the present study we had 12 cases of Bullous Pemphigoid (67%), 2 cases of CBDC (11%) We observed one case of dermatitis herpetiformis, linear IgA dermatoses, bullous SLE, LPP under the group of sub epidermal bullous disorders. Mucosal lesions, Nikolsky and Tzanck was negative in all cases. DIF was positive in 94.4% of the cases. The most common immune deposits were of IgG type and C3 followed by IgA and IgM. The deposits were in dermo-epidermal junction in 89% of cases, at papillary tip in dermatitis herpetiformis. The pattern of deposit was linear in 83%, and granular in 11%. However, the DIF was negative in LPP. The histopathology was positive in 83% of cases. DIF was diagnostic all the cases of Bullous Pemphigoid (100%). The immune deposits seen

were of IgG and C3 in all the cases. The deposits were in dermo-epidermal junction and the pattern of deposit was linear. Histopathology was positive in 92% of cases. DIF was diagnostic in 100% of the chronic bullous disease of childhood. The immune deposit was IgA type and positive in both cases. The deposits were in dermo-epidermal junction and the pattern of deposit was linear. Histopathology was positive in all cases. DIF was diagnostic in the dermatitis herpetiformis cases. The immune deposit was IgA type and the deposits was in papillary tip and the pattern of deposit was granular. However, Histopathology showed non-specific findings. DIF was diagnostic in the Linear IgA dermatoses case. The immune deposit was IgA type and the deposits was in dermo-epidermal junction and the pattern of deposit was linear. Histopathology was positive in the above case. DIF was diagnostic in the bullous SLE case. The immune deposits were positive for IgA, IgG, IgM and C3. The deposits were in dermo-epidermal junction and the pattern of deposit was granular. However, Histopathology showed non-specific findings. The clinical features, histopathology and DIF pictures of subepidermal bullous disorders are shown in Figures 3.

On comparision of Pemphigus Vulgaris (PV) and Bullous Pemphigoid (BP), the mean age in BP was 57 and in PV was 37. PV had female predominance (68%) and BP had male predominance (58%). Mucosal lesions, and Nikolsky sign were positive in all cases of PV and negative in BP. Tzanck was positive in 87% of cases of PV and negative in all cases of BP. DIF was diagnostic in 100% of both type of cases. Histopathology was positive in 86.7% of PV and 91.7% of BP.

In the present study, comparison between DIF and histopathology was done in both groups. Among Pemphigus group, DIF was found to be better than histopathology in the diagnosis of immunobullous disorders, though there was no statistical significance (p value :>0.5, Table1). Similarly, comparison was done in the subepidermal disorders and though there was no significant difference in the results, DIF proved to be better in the diagnosis of immunobullous disorders than histopathology.

DISCUSSION

Immunobullous disorders of skin are characterized by formation of autoantibodies against tissue proteins which lead to blistering of skin and mucous membrane. The most important techniques for the investigation of immunobullous disorders include conventional histopathology. Other tests that are essential as confirmative tests are Direct and Indirect immunofluorescence. In most of the immunobullous disorders, Clinical features and histological confirmation can make a correct diagnosis. Confirmatory studies such as immunofluorescence will help in diagnosis of cases where histopathological features alone are not diagnostic. This study was done to analyze the correlation of clinical features, histopathological findings and immunofluorescence in major immunobullous disorders.

In the present study, pemphigus Vulgaris was the most common immunobullous disorders which constituted about 43%. This correlated with the studies conducted by Arya SR et al.[4] and Chandrasekar et al studies. The next most common immunobullous disorder was bullous pemphigoid followed by CBDC. This was similar to studies conducted by Kanwar et al.[5] IgA pemphigus & Dermatitis herpetiformis was 3% which also correlated with Chandrasekar et al studies. Pemphigus Foliaceus was 3% in this study and the same was observed in the Arya SR et al and Chams-Davatchi et al studies.[6] Pemphigus group showed female preponderance and Subepidermal disorders showed a male preponderance which correlated with Micali et al.[7] and Aboobecker et al studies.[8] Nikolsky and Tzanck was positive in 87% of the cases similar to Handa F et al [9] studies. Histopathology was positive in 88% of the cases which was analogous to MM Huda et al studies.[10] DIF was positive in 100% of the cases, comparable to Kumar S et al studies.[11] The findings of Pemphigus Foliaceus correlated with studies done by Julie Leishangthem et al and Fernandez et al. Histopathology and DIF findings of IgA pemphigus was comparable with studies done by Nanda et al. In our present study bullous pemphigoid was seen in 34% which was analogous to Nanda et al studies. There was male preponderance which was also seen in Wong et al and Bernard P et al studies.[12,13] DIF was diagnostic in 100% of the cases Bullous Pemphigoid which was similar to Kippes W and Cozzani E et al studies.[14,15] In the present study 5% of cases were CBDC and the clinical, Histopathology and DIF correlated with the studies done be Chorzelsk et al.[16] DH constituted 3% of the study which was comparable to Chandrasekar et al studies. DIF was diagnostic in 100% of the cases as compared with Kambi et al studies. In LAD, DIF and Histopathology was 100% diagnostic which correlated with the studies done by Nurul Kabir et al and Wojnarowska et al studies.[17] DIF was diagnostic in bullous SLE, however Histopathology showed non-specific findings.

CONCLUSION

To conclude, in our study most frequent blistering disease were Pemphigus vulgaris, followed by Bullous pemphigoid. Females were largely affected by Pemphigus vulgaris, whereas, male predilection was seen in Bullous pemphigoid. While pemphigus vulgaris was found in middle aged, Bullous pemphigoid was seen in the elderly. Tzanck was positive in 87% of the cases in Pemphigus vulgaris which is slightly lower when compared to other studies, probably due to false negativity. Histopathology showed features correlating with respective disease in majority of the cases. DIF positivity in Autoimmune Bullous disorders was found in 97% cases. Intercellular space immune deposits (fish-net pattern) were the most frequent pattern encountered, followed by dermo-epidermal junction deposits (linear and granular pattern). DIF positivity was 100% for Pemphigus vulgaris, Bullous pemphigoid, Dermatitis herpetiformis, which is in accordance with

other studies. Clinical features correlated with DIF findings in 30 of 35 cases. The present study reaffirms that apart from new sophisticated tests (immunoblotting, immunoprecipitation, immunoelectron microscopy), the diagnosis of Autoimmune bullous disorders still relies on DIF findings in most laboratories. Thus, DIF still remains the gold standard and a cost-effective method in the diagnosis of blistering diseases. However, it is not a substitute to clinical or histopathological diagnosis but rather complementary to it. It is important to integrate clinical findings, histopathology and DIF in reaching an accurate diagnosis of Autoimmune bullous disorders.

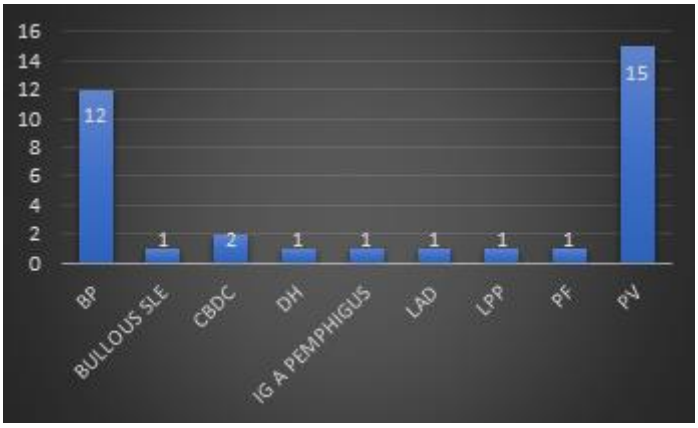

Figure 1: Distribution Of Immunobullous Disorders

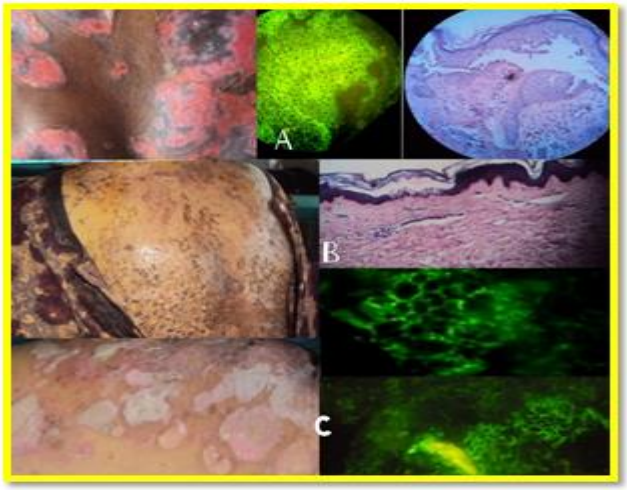

Figure 2: clinical, histopathology and DIF pictures of supra basal disorders- (a.)pemphigus vulgaris, (b.)pemphigus foliaceus, (c.)IgA pemphigus with fish net pattern

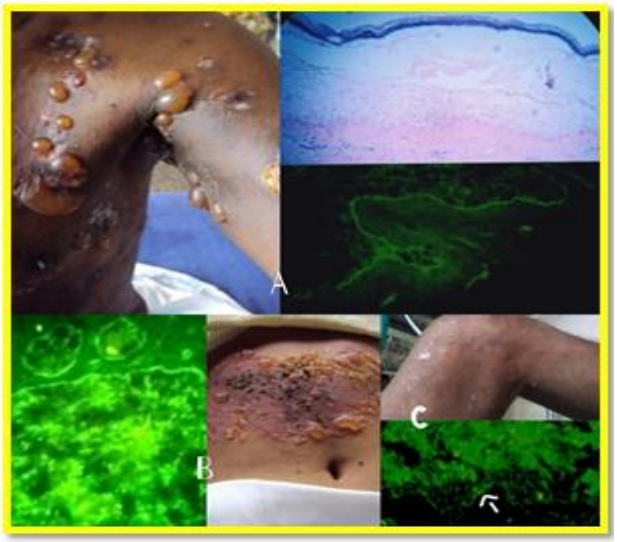

Figure 3: clinical, histopathology and DIF pictures of sub epidermal disorders- (a.)Bullous Pemphigoid, (b.)Bullous SLE, (c.) Dermatitis Herpetiformis

Table 1: comparison of histopathology and DIF findings

	Value	Df	Asymp. Sig.(2-sided)	Exact Sig.(2-sided)	Exact Sig.(1-sided)
Pearson Chi-Square	.212b	1	.645		

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