

# IMMUNOFLUORESCENCE IN DIFFERENTIATING BETWEEN BULLOUS PEMPHIGOID, EPIDERMOLYSIS BULLOSA ACQUISITA & CICATRICAL PEMPHIGOID OF BRUNSTING PERRY VARIANT.

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## ABSTRACT :

Direct and indirect immunofluorescence are helpful in diagnosing vesicobullous disorders. However, similar deposits along the basement membrane zone are seen in bullous pemphigoid (BP), cicatricial pemphigoid (CP) and epidermolysis bullosa acquisita (EBA). Immunofluorescence studies were performed in 3 cases of recurrent vesicobullous disorders with atypical clinical features by standard DIF, IIF, split skin techniques and by isopropyl alcohol fixation method. Diagnosis was confirmed by the ability of CP and BP to localise at the roof of split skin, and of EBA antigen to fluoresce the floor of split skin. The diagnosis of EBA & BP was further confirmed by loss of immunofluorescence in BP following the incubation of the substrate in isopropyl alcohol and continued fluorescence inspite of similar treatment in EBA. The fluorescence was seen to persist also in C P. Immunofluorescence using split skin techniques are helpful in differentiating subepidermal blistering disorders like BP, EBA & CP. Isopropyl alcohol fixation is an additional method to help in their differentiation.

Key words : Bullous pemphigoid, cicatricial pemphigoid Brunsting Perry variant,

Epidermolysis bullosa acquisita, Immunofluorescence.

## Introduction :

Vesicobullous disorders of autoimmune etiology resulting in subepidermal blister include bullous pemphigoid (BP), cicatricial pemphigoid (CP) and epidermolysis bullosa acquisita (EBA). We report a case of BP which was limited to oral mucosa, a case of CP which spared the oral mucosa and a case of EBA. The minor variations in immunofluorescence (IMF) techniques, which can help in the differentiation of the three conditions all of which show

linear IgG deposition along the basement membrane zone (BMZ) on routine IMF are explained.

## Patients & Methods :

**Case 1.** A 40 years old man presented with recurrent persistent oral erosions of eight years duration. As he failed to respond to conventional measures, biopsy for histopathology (H&E), and direct immunofluorescence (DIF) and serum for indirect immunofluorescence (IIF) were obtained. H&E section revealed a subepithelial bulla (Fig.1). DIF showed linear deposits of IgG along the BMZ. Normal human skin was split by incubating it in 1 M sodium chloride solution at 4 C for 48 hours (1) The IIF on intact skin showed deposits of IgG as a linear BMZ band with 1:10 and 1:80 dilutions of serum and split skin showed positive fluorescence on the under surface of the roof (Fig-2). Indirect immunofluorescence was also performed on 6 cm frozen section of normal human skin incubated in isopropyl alcohol for 30 minutes. This alcohol fixed section did not show the fluorescence (Fig-3). Immunoblotting detected a 230 kd band. Immunoelectronmicroscopy demonstrated antigenic location by gold labelling in the hemidesmosomes (Fig - 4 ).

**Case 2.** A 63 years old lady developed tense recurrent blisters which healed with scarring and occasional milia formation over upper trunk and scalp. Scalp revealed an area of cicatricial alopecia following blistering. Mucosal surfaces were spared. H & E examination revealed a subepidermal bulla (Fig- 5). DIF with IgG, IgA, IgM, C3 & fibrinogen were negative. IIF on normal skin gave a discontinuous linear IgG BMZ band. IIF on split skin detected IgG over the under surface of the roof of the blister at 1:10 and 1:20 dilution. The IIF on isopropyl alcohol fixed tissue also showed fluorescence on the roof of the split.

**Case.3** A 45 years old lady presented with recurrent tense blisters of one year duration. The blister which appeared on healthy skin healed normally with no visible scarring but occasional milia. There was no history of development of blisters following trauma. Biopsy for H & E was not done as there were no intact lesions. DIF showed deposits of IgG & C3 along BMZ. IIF on intact skin showed IgG

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Fig 1. Oral mucosal biopsy showing subepidermal blister in Bullous pemphigoid (BP) (Haematoxylin & eosin magnification x 10).

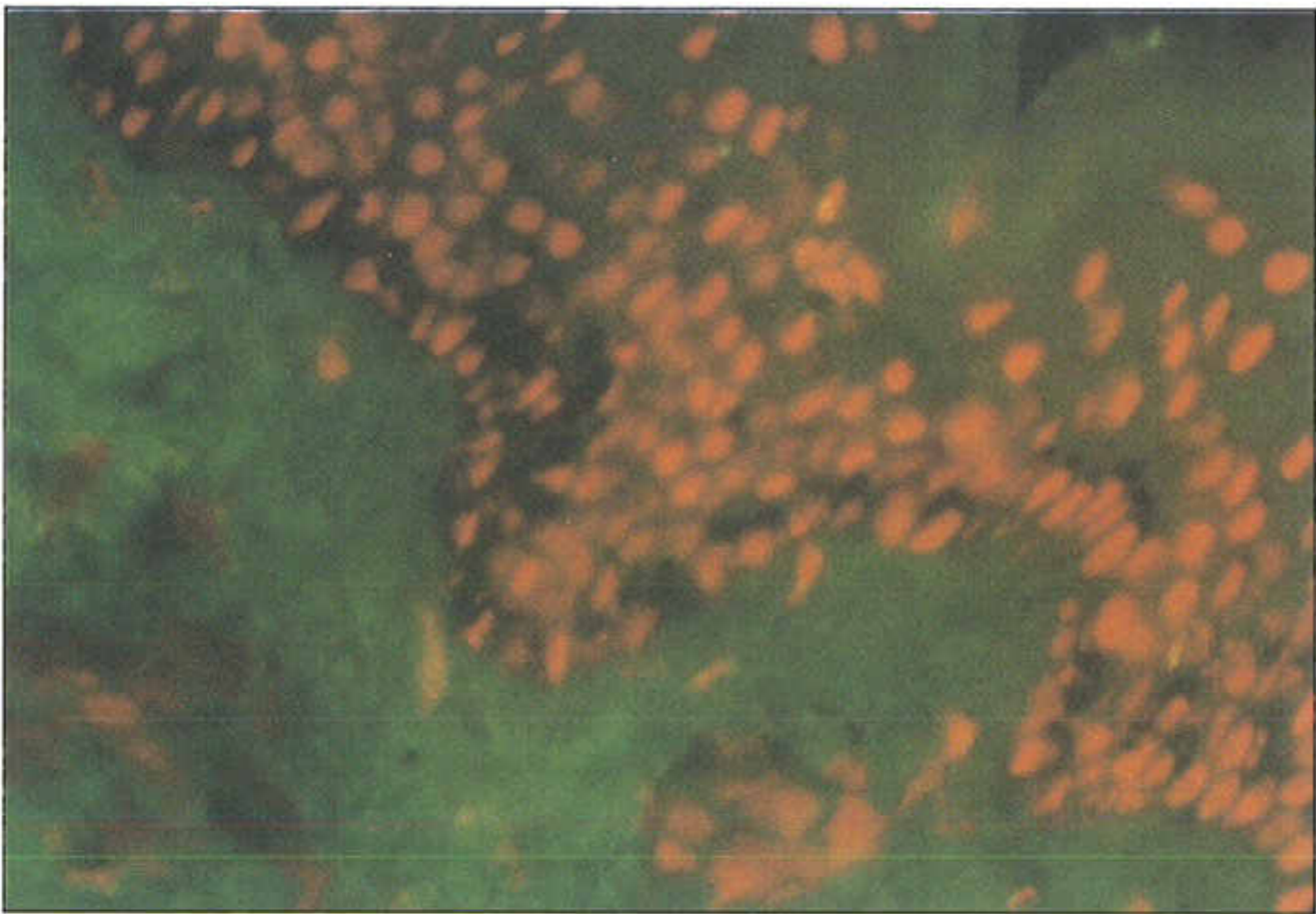


Fig 3. Indirect - Immunofluorescence with BP serum on Isopropyl alcohol fixed section showing loss of BMZ band.

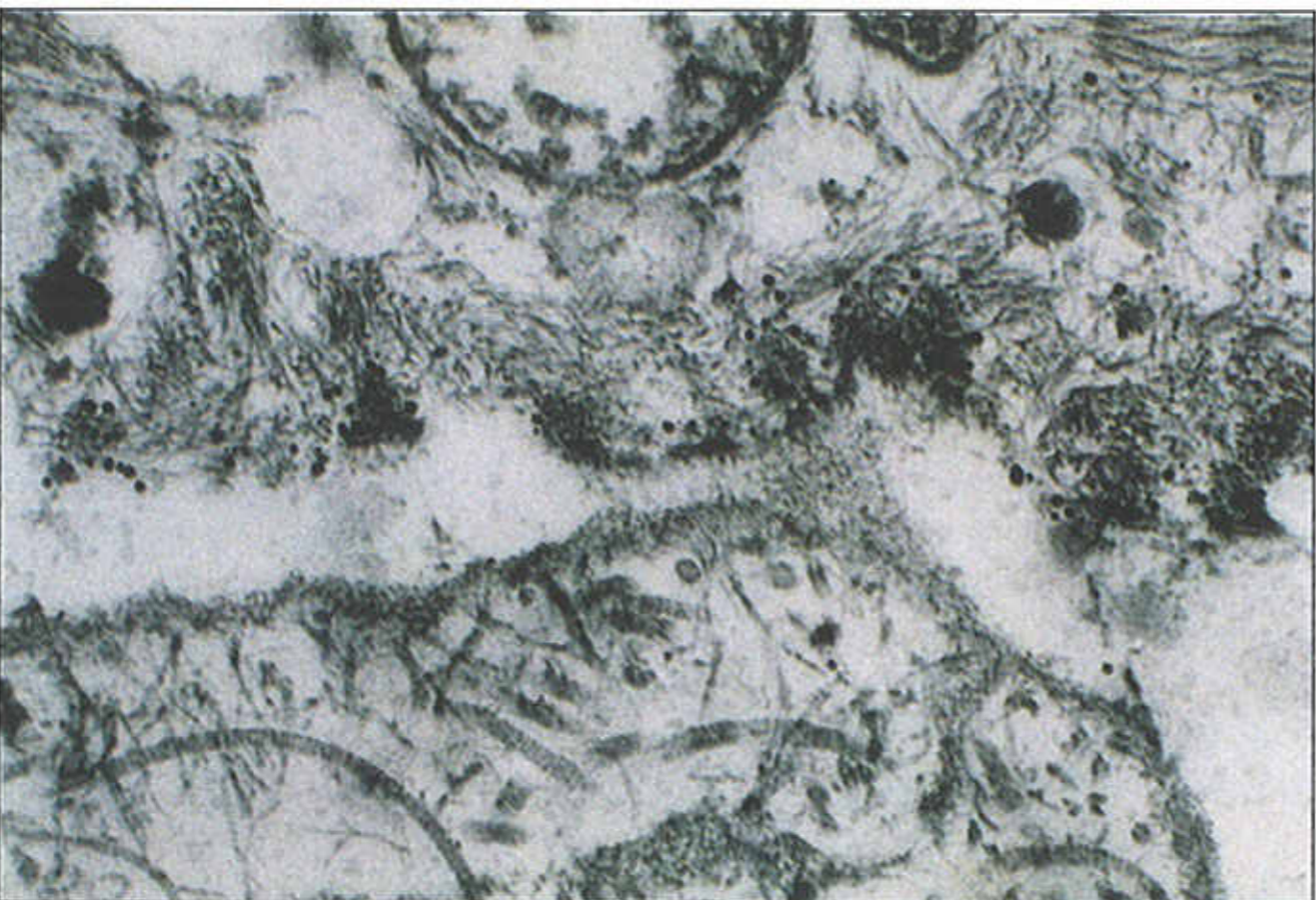


Fig 4. Immunoelectronmicroscopy showing gold labelling on hemidesmosomes in BP.

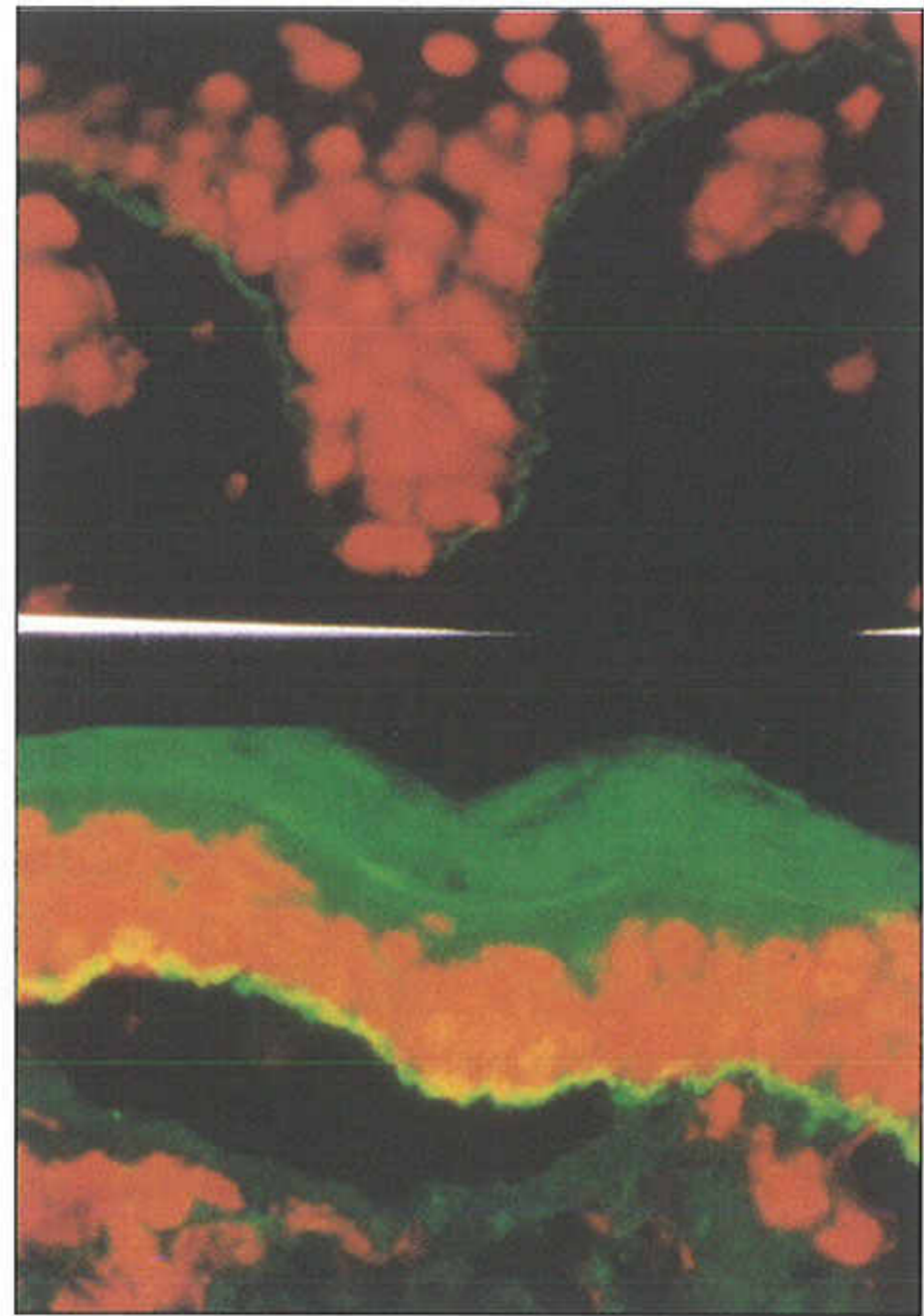


Fig 2. Indirect Immunofluorescence in BP. Upper half showing binding to the roof on split skin. Lower half showing IgG BMZ band.

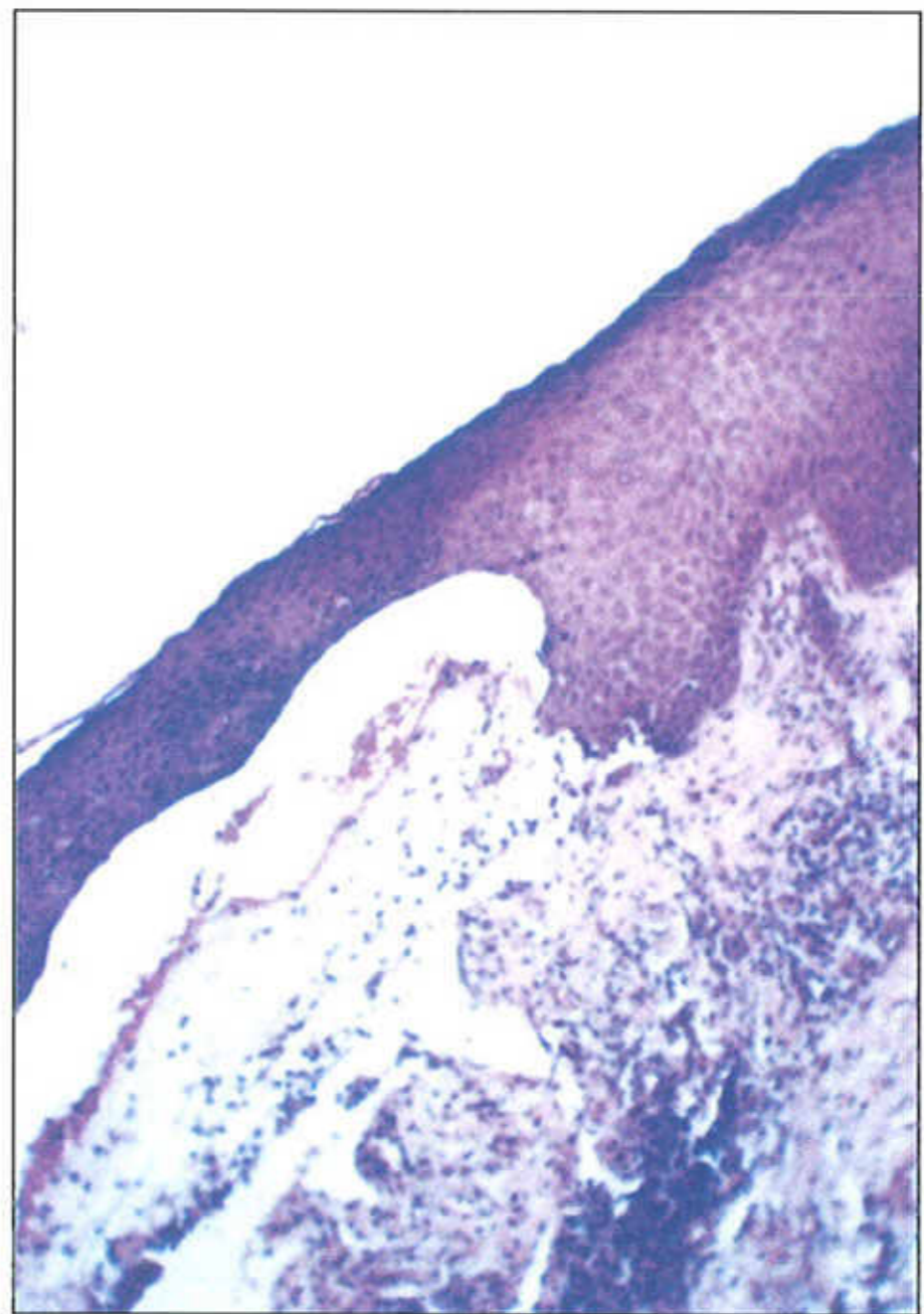


Fig 5. Skin biopsy showing subepidermal blister in CP of Brunsting Perry variant. (Haematoxylin & eosin magnification x 10 ).

band with 1:10 dilution and on split skin fluorescence at the floor (Fig-6). IIF done on isopropyl alcohol fixed tissue showed persistence of IgG BMZ band (Fig-7).

**Discussion :**

B P, C P & EBA have some clinical features, histopathological changes & IMF findings in common. The summary of the immunofluorescence findings is shown in Table 1. The diagnosis of these 3 cases was based on the following factors. B P is characterised by sub epidermal blister, IMF staining along the BMZ, positive fluorescence along the undersurface of the roof of split skin and the ability of ethyl alcohol to remove the B P antigen of normal skin on incubation<sup>(2)</sup>. Cicatricial Pemphigoid is characterised by sub epidermal blister, positive IMF along the under surface of the roof of split skin. The ability of C P to retain fluorescence following incubation in isopropyl alcohol is not reported. EBA is characterised by sub epidermal blister positive IMF along the floor of the split skin and ability to retain stain following incubation of the substrate in ethyl alcohol<sup>(2)</sup>. We however replaced ethyl alcohol with isopropyl alcohol for absolute alcohol is not licensed

by the Govt of India and not easily obtained. On correlating the above findings with clinical features we confirmed the first to be a case of BP limited to oral mucosa, the second a case of C P Brunsting - Perry variant involving upper trunk and scalp, sparing mucosa, and healing with scarring and milia<sup>(3)</sup> and the third as a case of EBA.

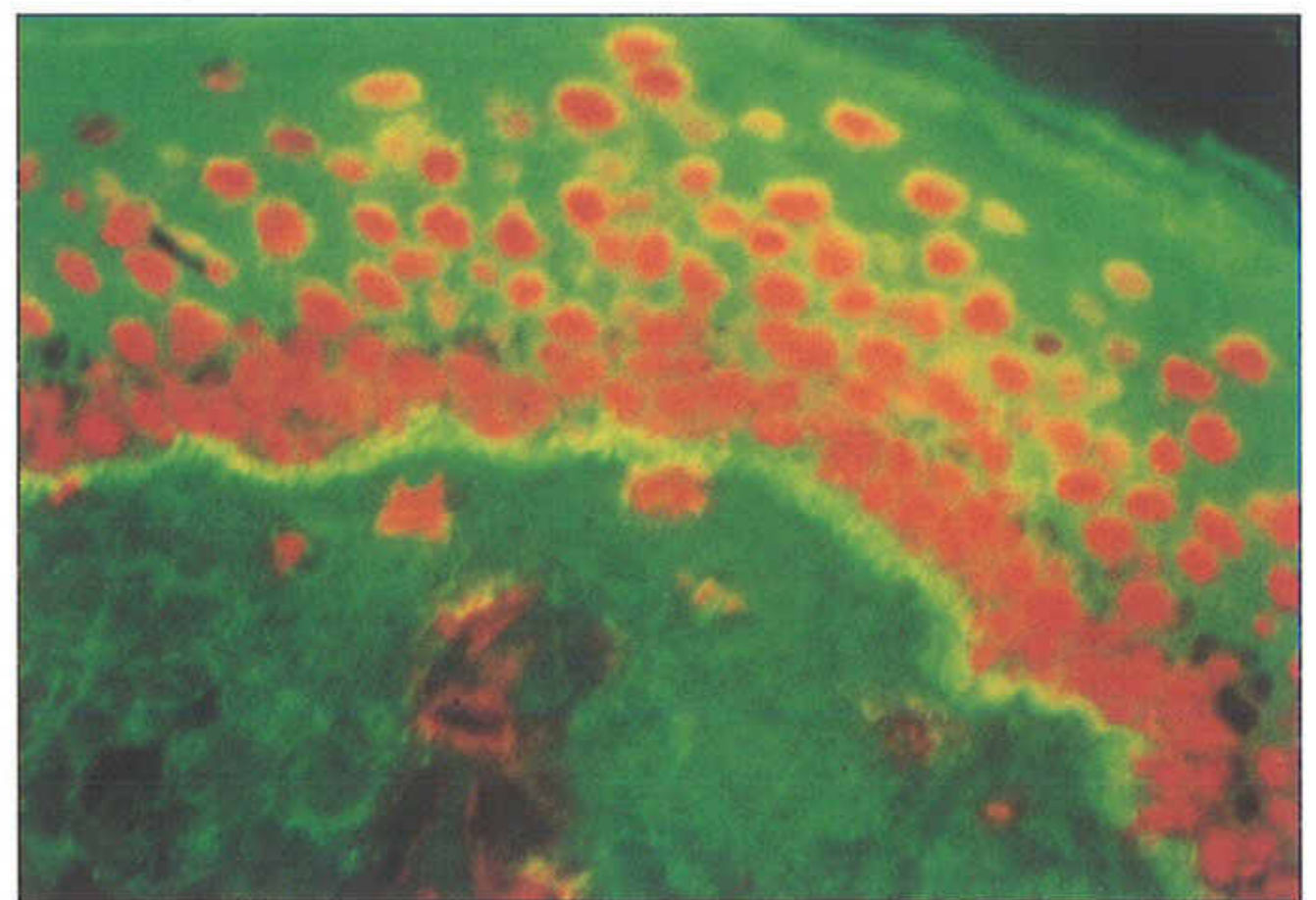


Fig. 7. Indirect Immunofluorescence with EBA serum on Isopropyl alcohol fixed section. The IgG BMZ band being retained

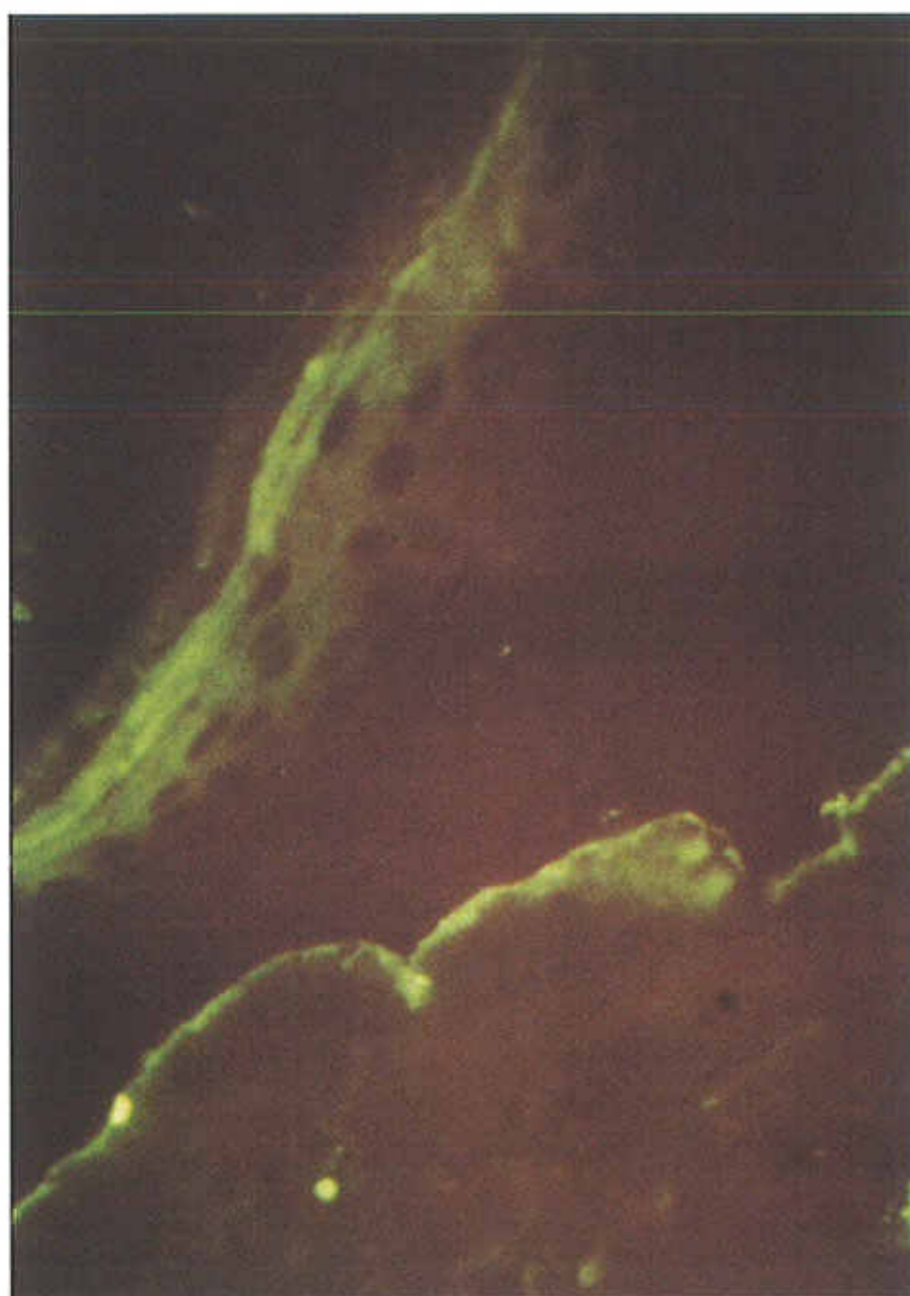


Fig 6. Indirect Immunofluorescence with EBA serum on split skin showing fluorescence at the floor.

Table 1 : Results

Case No.	Diagnosis	DIF	IIF	Split Skin IIF	IIF on Alcohol Fixed Skin
1	ORAL BP	Positive	Positive	Roof	Negative
2	CP Brunsting perry	Negative	Negative	Roof	Positive
3	EBA	Positive	Positive	Floor	Positive

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