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Original Research Article

Periodic acid-schiff staining: A counterpart of the immunoreactivity seen by direct immunofluorescence in bullous pemphigoid

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ABSTRACT

Background: Periodic-Acid-Schiff (PAS) stain has been put forward to differentiate between bullous pemphigoid (BP) and epidermolysis bullosa acquisita (EBA). PAS stain shows correspondence with DIF staining patterns of EBA and BP with respect to basement membrane staining. Histological hall-mark of this entity is subepidermal blister containing eosinophils. But there can be other findings observed in this entity which can cause pitfalls in the diagnosis. Our study demonstrates these rare findings to avoid the misdiagnosis.

Aims and Objectives: To study the utility of Periodic-acid-Schiff stain in differentiating BP and EBA on histopathology. To evaluate 'n' versus 'u' serration patterns in differentiating BP and EBA on DIF examination.

Materials and Methods: Two skin punch biopsies measuring 3 mm were obtained. Of the two skin punch biopsies, one was sent in Michel's medium for direct immunofluorescence (DIF) and the other for light microscopy. PAS stain was performed on light microscopy.

Results: 18 (100%) clinically suspected cases of BP were assessed for light microscopy and DIF, of which 16 (88%) cases were confirmed by DIF examination and histopathology. 16 of them (88%) showed both floor pattern and basal fraying of keratinocytes. 4 (25%) cases showed a unique finding of linear arrangement of neutrophils along the basal layer of epidermis with subepidermal bulla containing eosinophils.

Limitations: Small sample size, inability to apply population based statistics and absence of the comparable group (EBA) due to which the findings may not be specific to the disease entity.

Conclusions: PAS stain can be used to provide simple, cost-effective and reliable diagnosis in differentiating BP versus EBA cases, although indirect immunofluorescence remains the gold standard test for confirmation. BP should be differentiated from other subepidermal diseases to avoid the diagnostic pitfalls and misdiagnosis of the same, as neutrophils can also be present in BP as the main inflammatory cells and share some similar histological features with the other subepidermal diseases.

1. Introduction

Bullous pemphigoid (BP) is an autoimmune subepidermal vesiculobullous (VB) disorder of the elderly. This disease entity is difficult to distinguish from epidermolysis bullosa acquisita (EBA) and exhibit similar features on histology as well as on direct immunofluorescence (DIF).[1] Periodic-Acid-Schiff (PAS) stain has been put forward to differentiate between BP and EBA. PAS stain shows correlation with DIF staining patterns of EBA and BP with respect to basement membrane staining patterns and basal fraying of keratinocytes.[2,3]

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Bullae formation in BP is mediated through autoantibodies against BP antigen (BP180) and BP antigen 230 (BP230).[4,5] In EBA, bullae formed are mediated through autoantibodies against type VII collagen, a prime component of anchoring fibrils in BMZ which connects the dermis to the BMZ. We evaluated the utility of PAS stain and 'n' versus 'u' serration patterns in differentiating BP and EBA on histopathology and DIF examination respectively.[6]

The normal basement membrane is highlighted by PAS stain. Autoantibodies against BP induce a cleft in the upper part of epidermis in BP, due to which the bulla is formed towards the dermis, pushing the BM complex forming a floor pattern.[7,8] Contrarily, EBA shows BM complex localising towards epidermal side, exhibiting a roof pattern. The presence or absence of fraying of keratinocytes is also another feature used to differentiate between BP and EBA. Disruption of intracellular BP180 and BP230 causes damage to the basal keratinocytes, hence the basal fraying of keratinocytes are more observed in BP. In EBA, there is absence of fraying of keratinocytes as they are still attached to the BM towards the epidermal side of the split.[5]

We explicated that PAS staining of BM pattern and presence or absence of basal fraying of keratinocytes can help in discerning BP and EBA.[6]

2. Materials and Methods

The present study is a time bound, record based retrospective and prospective study in the department of Pathology, Kasturba Medical College, Mangalore from October 2018 to March 2021. All skin biopsy specimens, clinically suspected to be BP and EBA cases, received for histopathology and DIF were studied and analysed. Inadequate samples were excluded from the study. The clinical and laboratory data pertaining to clinical features, anatomical site, size of the lesion and associated symptoms were collected from the biopsy requisition forms. The study was approved by Institutional Ethics Committee of Kasturba Medical College, Mangalore (IEC KMC MLR 09-19/388)

Two skin/ mucosal biopsies measuring 3 mm were obtained by punch biopsy from each patient. Of the two skin punch biopsies, one biopsy was sent in Michel's transport medium for DIF and the other biopsy in 10% neutral buffered formalin (NBF) for light microscopy and special stain: PAS was done on all routine microscopy slides.The tissue sent in Michel's medium was frozen and processed for DIF examination (IgG, IgA, IgM, C3 and fibrinogen).

The tissue obtained in 10% NBF was processed and embedded in paraffin wax and cut 3 – 5 micron thickness sections and stained using hematoxylin and eosin (H & E) for light microscopy. The routinely stained slides and DIF examination slides were reviewed to confirm the histology and immune deposition of markers characteristic of BP. The data was tabulated in the excel sheet and the results were analysed in percentages and frequencies. The intensity of staining in DIF examination was graded from 0 to 3+, 0 - no staining, 1+ weak/faint, 2+ moderate and 3+ strong.[9]

3. Results

A total of 18 (100%) clinically suspected cases of BP were received for DIF and light microscopy examination, of which 16 (88%) cases were diagnosed and confirmed by DIF examination and 14 (77%) cases were confirmed on light microscopy as BP. Of these 16 cases, males (69%) were more commonly affected than females (31%). Majority were in the age group between 41 – 95 years, mean age being 71years. These cases presented mainly with tense bullae in 12 cases (75%) involving whole of the body. On histopathological examination, all the 16 (100%) showed a subepidermal blister with 9 cases (64.3%) of cell-rich variant and 5 cases (35.7%) of cell-poor variant. Blister content was composed of predominantely eosinophils in 13 (81%), predominantely neutrophils in 2 (13%), lymphocytes in 4 (25%) and few neutrophils in 9 (56%) cases.

PAS stain findings showed prominent floor pattern of staining and basal fraying of keratinocytes present in all the 16 cases (100%) respectively.

Epidermal changes included the most common epidermal findings like spongiosis in 10 cases 63%) and eosinophilic spongiosis in 4 (25%). We also noted eosinophilic tagging in 7 (44%), acanthosis in 9 (56%) cases and a rare, infrequent finding of linear arrangement of neutrophils in 4 cases (25%). Dermal changes mainly showed dermal edema in 14 cases (88%), eosinophilic microabscesses in 5 (31%) and neutrophilic microabscesses in 3 cases (19%). Perivascular inflammatory infiltrate was composed predominantely of eosinophils in 5 cases (31%), predominantely neutrophils in 2 (13%), predominantely lymphocytes in 6 (38%), and mixed inflammatory infiltrate in 7 cases (43%).

DIF examination revealed 14 cases (87.5%) with IgG deposition and 16 cases (100%) with C3 deposition. The pattern observed was linear continuous positivity along the basement membrane zone. We also observed a serration pattern on DIF which showed "n" serration pattern in 16 cases (100%). All the above observed findings confirmed the diagnosis of BP.

4. Discussion

PAS staining highlights the BM complex and the staining was done in the cases of BP to see the pattern of BMZ localization and the presence / absence of basal fraying of keratinocytes. Sixteen cases (100%) showed floor pattern of staining and presence of basal fraying of keratinocytes. Two cases were inconclusive on HP, which were later proven on DIF as BP. In BP, autoantibodies BP180 and BP230 induce a cleft more on the superficial side of the BM, hence the

Table 1: Epidermal changes in BP

Subepidermal bulla	Blister content – predominantely eosinophils	Cell-rich	Cell-poor	Linear arrangement of neutrophils	Eosinophilic tagging	Eosinophilic spongiosis
16 (100%)	13 (81%)	9 (64.3%)	5 (35.7%)	4 (25%)	7 (44%)	4 (25%)

Table 2: DIF findings with distribution of patterns in BP

Diagnosis	Location of deposits	Nature of deposits	Pattern	Extent	Intensity
BP	BMZ – 16 (100%)	Ig G – 14 (87.5%)	Linear BMZ	Continuous throughout BMZ	1+ - 1 (6%)
DIF positive – 16 n – 18		C3 – 16 (100%)			2+ - 5 (31%) 3+ - 10 (63%)

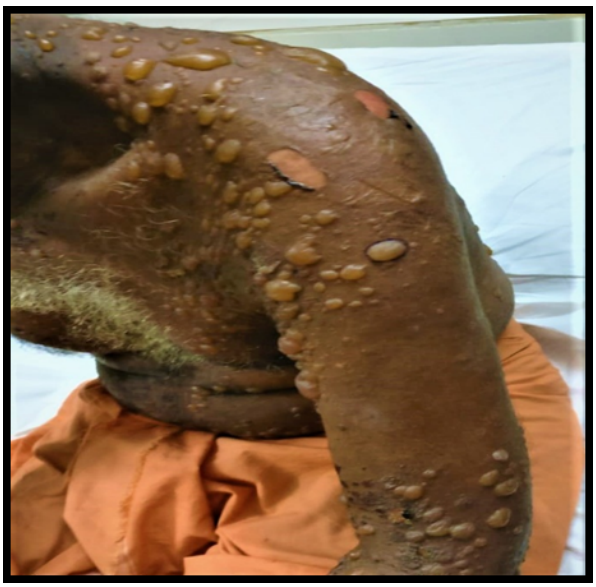

Figure 1: Case of BP showing tense bullae and vesicles on the trunk, chest and upper limbs

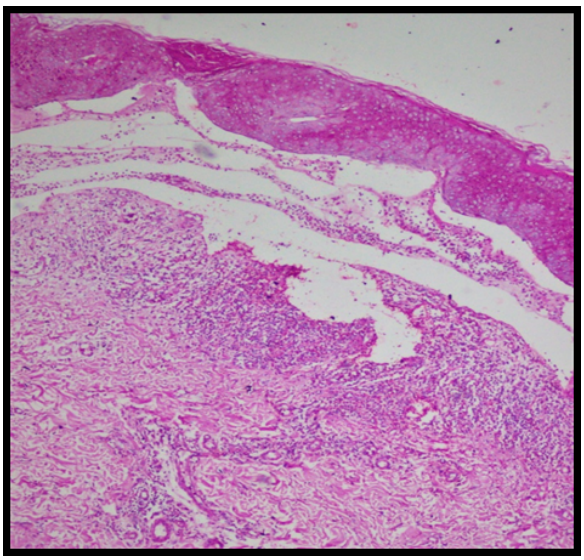

Figure 2: BP showing a subepidermal blister, with cell-rich inflammatory infiltrate composed predominantely of eosinophils and a few neutrophils in the dermis and bullous cavity (H&E, x100)

BM formed localizes predominantely on the floor of the bulla (dermal side). Conversely, the BM localizes towards the epidermal side in EBA as the antibodies against type VII collagen induce a cleft on the deep side of BMZ.

The basal fraying of keratinocytes in BP is due to the damage caused by destruction of the intracellular portion of BP230 and BP180 antigen as the keratinocytes are pulled apart when the bullae are formed. There are few cases of EBA where BM localizes towards the floor as PAS stains lamina densa along with type VII collagen and other components that are on the dermal side of BMZ. The deeper components take up the stain and persist on the dermal side following the pathologic split of type VII collagen, similarly, the split can occur more superficially at lamina densa due to various factors like structural integrity and destructive mediators of inflammation.[10–12]

Gardner K et al[13] in their studyreviewed 13 EBA and 19 BP cases of DIF confirmed cases. These cases were assessed on PAS stain for the BM patterns and basal fraying of keratinocytes in EBA, which showed roof staining with sensitivity 25% and specificity 95% with no keratinocyte fraying with sensitivity 62% and specificity 58%. Eighteen cases of BP showed floor staining and 11 cases of BP showed fraying of keratinocytes. These findings are consistent with our study findings along with other studies.

BP is traditionally classified under predominantely "subepidermal blistering disease with eosinophils" as the main inflammatory infiltrate in contrast to "subepidermal disease with neutrophils".[14] Although most of our cases showed eosinophil rich interstitial, dermal and perivascular infiltrate, some of our cases even showed neutrophils present

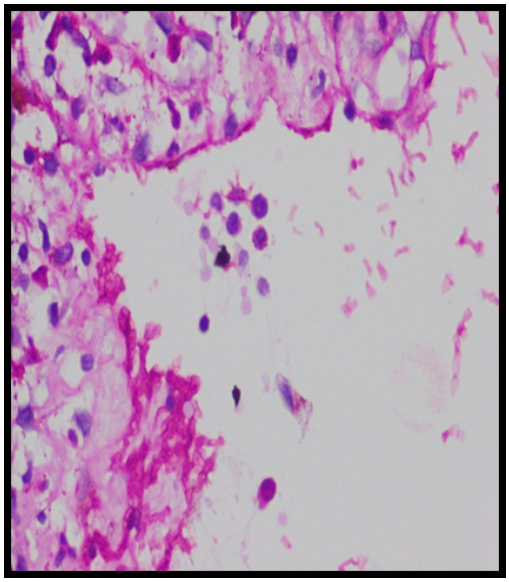

Figure 3: PAS stain findings of BP showing basal fraying of keratinocytes and basement membrane localisation towards the floor (H&E, x400).

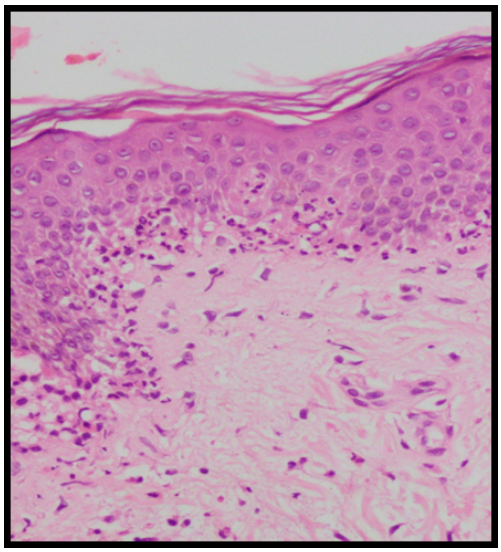

Figure 4: BP showing a unique feature - linear arrangement of neutrophils along the basal layer of epidermis (H&E, x100)

in a linear pattern below the basal layer of epidermis illustrating the heterogenecity of the histological findings of BP. However we did not have any confirmed cases of EBA to analyse and compare the above mentioned findings. There is scope to gather more accurate findings with a larger sample size with the cases of comparable group.

A case report by Andrachuk et al[15] studied a clinically suspected case of BP in a 59 year old female who presented with chief complaints of pruritic blisters and eruptions. Biopsy findings revealed subepidermal blister

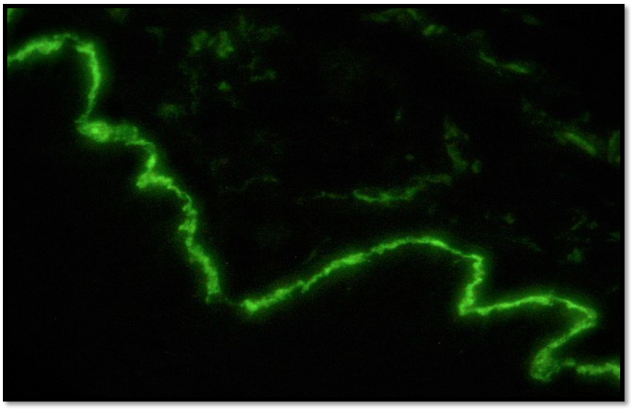

Figure 5: DIF image of BP showing continuous linear deposition of IgG and C3 along the BMZ (DIF, x100)

with inflammatory infiltrate composed of lymphocytes and neutrophils in superficial dermis. They also noted a rare feature - linear pattern of neutrophils observed beneath the basal layer in the epidermis. This finding was similar to the histology seen in linear IgA disorder. Our cases also showed similar findings concordant with their case report.

Min et al[16] reported a 71-year-old male with chief complaints of plaques and erythema on the extremities and trunk for the past 3 months along with itching. Histopathology of the same revealed a subepidermal blister with eosinophils, lymphocytes and few neutrophils. Superficial dermis showed focal and dense eosinophilic and lymphocytic perivascular inflammatory infiltrate. In addition to these findings, they also found neutrophils present in a linear pattern beneath the basal layer. This case was confirmed by DIF examination which showed linear pattern of C3 and IgG positivity along the BMZ. BP180 levels of 30.99 U/mL was noted in ELISA test.[5] Combining clinicopathlogical and DIF examination findings, a diagnosis of BP was made, which showed concordance with our case series.

There is limited literature on neutrophil predominant inflammatory infiltrate and almost finite reports on linear arrangement of neutrophils along BMZ in BP.[17] This feature mimicks the histological findings of linear IgA dermatosis and EBA. We confirmed all the BP cases by DIF examination which revealed linear diffuse IgG and C3 positivity along the BMZ and we studied the serration pattern to differentiate between BP ("n" serration pattern) and EBA ("u" serration pattern).[18] All our cases demonstrated "n" serration pattern in favor of BP. Although we did have one clinically suspected case of EBA, DIF examination was negative.

We had a small sample size and no comparable group (EBA) in this study, hence, the above mentioned findings on PAS stain needs to be studied in the cases of EBA.

Limitations of the study included small sample size, inability to apply population based statistics and absence of the comparable group (EBA) due to which the findings may not be specific to the disease entity.

5. Conclusion

The histological differences between BP and EBA disorder should be analysed to avoid the diagnostic pitfalls and misdiagnosis of the same. Neutrophils present in a linear pattern beneath the basal layer of epidermis in a case of BP should not be incorrectly diagnosed as other disease entities.

PAS stain along with 'n' versus 'u' serration pattern helps us in distinguishing EBA from BP and other pemphigoid group of disorders on DIF. Further, studies in correlation with IIF may be which helps to determine the significance of the findings comparing BP and EBA may be considered.

6. Sources of Funding

None.

7. Conflict of Interest

The authors have no conflicts of interest.

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