



HISTOMORPHOLOGICAL ANALYSIS OF SUBEPIDERMAL BULLOUS DISORDERS OF SKIN BY PERIODIC ACID-SCHIFF STAIN

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Abstract

Background: Subepidermal bullous illnesses are autoimmune blistering diseases that exhibit notable clinicopathological overlap and separation at the dermoepidermal junction. For proper diagnosis and treatment, accurate distinction is crucial. The basement membrane zone can be highlighted by Periodic Acid–Schiff (PAS) stain, which can help determine the degree of separation.

Aim: Examine the histomorphological characteristics of subepidermal bullous diseases and assess the diagnostic value of PAS staining.

Materials and Methods: As part of this prospective investigation, 40 skin samples from clinically suspected bullous disorders were assessed over a one-year period using PAS and Hematoxylin and Eosin staining. Histomorphology, PAS staining patterns, and inflammatory infiltration were evaluated and linked to the diagnosis.

Results: With a female preponderance, bullous pemphigoid was the most prevalent entity (65%). Subepidermal blisters formed in all patients with the most common pattern being an eosinophil-rich infiltration (55%). In every instance, PAS staining brought attention to the basement membrane zone displaying a floor pattern in 80% of cases and a roof pattern in 20%. Whereas the roof pattern was observed in porphyria cutanea tarda, bullous lupus erythematosus, and epidermolysis bullosa

acquisita, floor staining was primarily associated with bullous pemphigoid and lichen planus pemphigoides.

Conclusion: In conclusion, PAS provides a straight forward and reasonably priced addition to standard histology for the differentiation of subepidermal bullous diseases.

Keywords: Subepidermal Bullous Disorders, Histomorphology, PAS Stain, Dermatopathology, Basement Membrane Zone, Bullous Pemphigoid, Skin Biopsy, Histopathology

Introduction

The subepidermal autoimmune bullous diseases are a diverse category of chronic blistering conditions that are typified by the production of subepidermal blisters and separation at the dermoepidermal junction due to autoantibody-mediated destruction to structural elements of the basement membrane zone. Bullous pemphigoid, epidermolysis bullosa acquisita, and other associated conditions are among the illnesses that exhibit significant clinicopathological and epidemiological heterogeneity among other groups (1). Autoimmune subepidermal blistering illnesses frequently manifest after the age of 60 to 70 years, however they can also afflict infants and pregnant women. They exhibit significant clinical and immunopathological overlap. (2) Chronic erosions and scarring can occur when lesions primarily impact or are restricted to mucous surfaces, such as the oral and ocular mucosa. In other situations, skin lesions typically appear during pregnancy. Lastly, blistering and skin fragility over traumatized areas result in skin shrinkage and scarring in other patients. While the disease usually manifests as a widespread blistering eruption accompanied by itching, atypical variations with either localized bullous lesions or non-bullous presentations are seen in about 20% of patients and pose a diagnostic challenge. The quality of life is significantly impacted by BP because of the intense itching and skin lesions, and it also has a high death rate. Between 2.4 and 21.7 new cases of BP are reported for per million people annually(3-4). Although a combination of clinical characteristics and direct and indirect immunofluorescence on salt-split skin can be used to diagnose BP, histopathology is typically carried out to confirm BP and rule out other possible causes. Among the clinical characteristics that support a BP diagnosis are age >70; lack of head and neck blistering; lack of mucosal involvement; and lack of atrophic scarring (5-6). According to European statistics, the mortality risk is highest during the first year following diagnosis, with rates ranging from 29 to 41%. (7) Histopathologically, pemphigus is defined by the production of intraepidermal blisters due to keratinocytes' lack of intercellular adhesion (acantholysis), which usually results in suprabasal clefting with acantholytic cells inside the blister cavity. The loss of basal keratinocyte adherence to the basement membrane zone, on the other hand, causes subepidermal blister formation in bullous pemphigoid and similar pemphigoid illnesses. Hemidesmosomal antigens at the dermoepidermal junction are implicated in the inflammatory subepidermal split caused by autoantibody-mediated complement activation in pemphigoid, which has an intact epidermal roof and an underlying dermal inflammatory infiltration (8). Clinical manifestations vary according to the organs that the immune response targets, and the pathophysiology is frequently complex, incorporating environmental triggers as well as hereditary predisposition (9). The staining technique known as periodic acid-Schiff (PAS) is used to identify polysaccharides, which can be found in tissues as mucin, glycogen, glycoproteins, and glycolipids . Generally speaking, tissues treated with 10% neutral buffered formalin are the best candidates for the PAS stain. (10, 11). The level of the split in subepidermal bullous diseases could be defined using the periodic acid-Schiff (PAS) stain's ability to show basement membrane. The ability of PAS to highlight the basement membrane is mostly attributed due to the basement membrane constituents that take up PAS stain, especially laminin and type IV collagen. Blistering in EBA is below the basement membrane, whereas blistering in BP is superficial to the basement membrane. As a result, the ability of PAS to stain the blister's floor in BP and roof in EBA may be used to differentiate the two entities. (9) Thus, the goal of the current study was to analyze the histomorphological characteristics of subepidermal bullous disorders and assess how well Periodic Acid-Schiff (PAS) staining works to highlight the basement membrane zone and indicate the degree of split, which will help distinguish

between different subepidermal bullous lesions as an easy and affordable addition to standard histopathology.

Materials and Methods

This prospective observational study was carried out in the Department of Pathology at ACS Medical College and Hospital from February 2025 to February 2026. A total of 40 skin biopsy specimens were included based on statistical sample size estimation. The study population comprised patients above 18 years of age, both males and females, who presented with a clinical suspicion of bullous disorders. Relevant clinical details, including age, gender, and provisional diagnosis, were documented.

All biopsy specimens were fixed in 10% neutral buffered formalin and subjected to routine tissue processing and paraffin embedding. Sections of 3–5 μ m thickness were prepared and stained with Hematoxylin and Eosin (H&E) for detailed histomorphological evaluation. Periodic Acid–Schiff (PAS) staining was performed to highlight the basement membrane zone and to determine the level of split at the dermoepidermal junction. Histopathological parameters assessed included the level of blister formation, nature of inflammatory infiltrate, and associated epidermal and dermal changes. The PAS staining pattern was categorized as floor or roof staining and correlated with the histopathological diagnosis. Cases with inadequate tissue, improperly fixed specimens, or patients already on treatment for bullous disorders were excluded to ensure accurate interpretation.

Results:

Table 1: Gender distribution

Gender	Frequency	Percentage (%)
Female	23	57.5
Male	17	42.5

The gender distribution of study participants is displayed in Table 1. Of the total number of cases, 17 (42.5%) were male and 23 (57.5%) were female. This suggests that there is a small female preponderance in the research population.

TABLE 2: Distribution of Subepidermal Bullous Disorders (n = 40)

Diagnosis	Frequency	Percentage (%)
Bullous Pemphigoid	26	65
Lichen Planus Pemphigoides	5	12.5
Epidermolysis Bullosa Acquisita	4	10
Bullous Lupus Erythematosus	3	7.5
Porphyria Cutanea Tarda	2	5

The distribution of the several subepidermal bullous diseases found in the 40 cases in this investigation is displayed in the table. with 26 instances (65%), bullous pemphigoid was the most common diagnosis, clearly showing its preponderance in the study population.

With five occurrences (12.5%), lichen planus pemphigoides was the second most common entity. Four instances (10%) had epidermolysis bullosa acquisita, while three cases (7.5%) had bullous lupus erythematosus. With two instances (5%), porphyria cutanea tarda was the least prevalent condition in this investigation.

TABLE 3: Nature of Inflammatory Infiltrate on H&E (n = 40)

Inflammatory Infiltrate	Frequency	Percentage (%)
Eosinophil-rich	22	55
Neutrophil-rich	11	27.5
Cell-poor	7	17.5

The distribution of inflammatory infiltration patterns seen in 40 instances of subepidermal bullous diseases is displayed in the table. The most frequent pattern, observed in 22 instances (55%), was an eosinophil-rich infiltration, suggesting that in over half of the cases, eosinophils were the major inflammatory cell type. 11 cases (27.5%), or roughly one-fourth of the study group, had neutrophil-rich infiltrates. 7 instances (17.5%) had a cell-poor inflammatory pattern, which is the least frequent presentation.

TABLE 4: PAS Staining Pattern (n = 40)

PAS Pattern	Frequency	Percentage (%)
Floor	32	80
Roof	8	20

The distribution of PAS staining patterns among 40 cases of subepidermal bullous diseases is displayed in Table 4. While 8 cases (20%) showed staining on the blister roof, 32 cases (80%) showed PAS positive along the blister floor. The basement membrane zone was typically kept on the cutaneous side of the split, as seen by the prevalence of floor stains. This pattern helps determine the degree of cleavage and supports the histological diagnosis of subepidermal blistering disorders.

TABLE 5: Correlation Between Diagnosis and PAS Pattern

Diagnosis	Floor	Roof	Total
Bullous Pemphigoid	26	0	26
Lichen Planus Pemphigoides	5	0	5
Epidermolysis Bullosa Acquisita	1	3	4
Bullous Lupus Erythematosus	0	3	3
Porphyria Cutanea Tarda	0	2	2

Based on the degree of immune deposition at the split's floor and roof, the table displays the distribution of subepidermal bullous diseases. The most frequent diagnosis (26 instances) was bullous pemphigoid, and all of the patients showed floor deposits but none at the roof. Likewise, floor pattern deposition was observed in all five lichen planus pemphigoides instances. On the other hand, only one case of epidermolysis bullosa acquisita showed floor deposition, while three cases mostly displayed roof pattern deposition. Porphyria cutanea tarda (2 instances) and bullous lupus erythematosus (3 cases) all showed unique roof pattern deposition.



Figure 1: Clinical photograph showing a case of bullous pemphigoid with multiple ruptured bullae present over the trunk, chest, and upper limb.

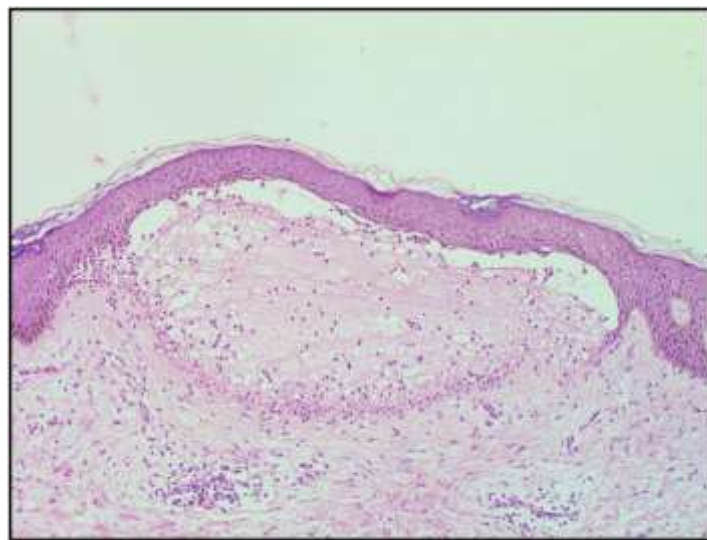


Figure 2: Histopathological image (H&E, 4×) showing a subepidermal blister.

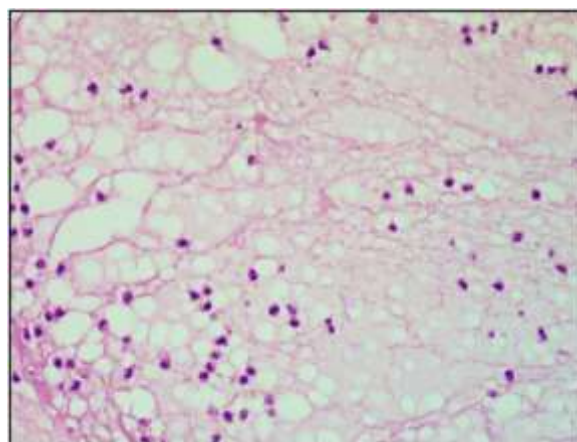


Figure 3: Higher magnification view demonstrating the blister cavity containing numerous inflammatory cells, predominantly eosinophils along with neutrophils.

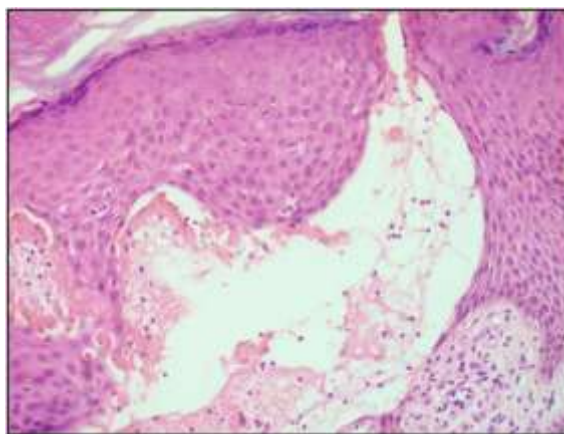


Figure 4: Histopathological section showing spongiosis in the epidermis adjacent to the blister

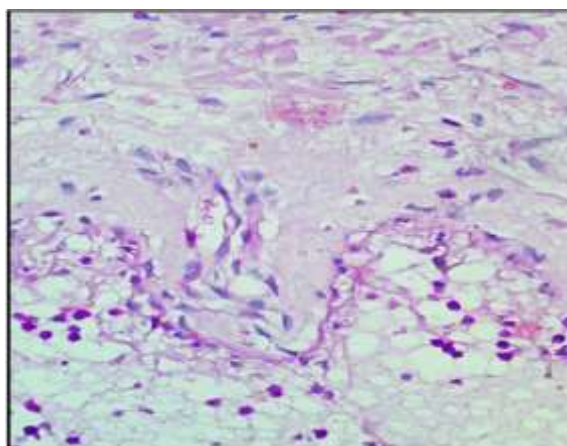


Figure 5: Periodic Acid–Schiff (PAS) stain highlighting the basement membrane zone along the floor of the bulla in bullous pemphigoid.

Discussion

In the present prospective study of 40 cases, bullous pemphigoid was the most common entity (65%) with a female predominance (57.5%), and all cases showed subepidermal blister formation on histopathology. Eosinophil-rich inflammatory infiltrate was the predominant pattern, observed in 55% of cases and most frequently associated with bullous pemphigoid. PAS staining highlighted the basement membrane zone in all cases, with a floor pattern seen in 80% of cases, particularly in bullous pemphigoid and lichen planus pemphigoides. Roof pattern staining was mainly noted in epidermolysis bullosa acquisita, bullous lupus erythematosus, and porphyria cutanea tarda, indicating PAS as a useful adjunct diagnostic tool.

Ponmar et al. (2024) reported bullous pemphigoid as the most common subepidermal bullous disorder with subepidermal blister and eosinophil-rich bulla, and found that PAS staining showed a floor pattern in bullous pemphigoid and lichen planus pemphigoides, while roof pattern was seen in bullous SLE and porphyria cutanea tarda. Similarly, the present study also demonstrated predominance of bullous pemphigoid, subepidermal blister formation, eosinophil-rich infiltrate, and predominant floor pattern PAS staining, supporting the use of PAS as a valuable adjunct to routine histopathology in differentiating subepidermal bullous disorders.(12)

The Abreu Velez AM et al (2016) of a study comparing PAS and direct immunofluorescence (DIF) staining in a variety of autoimmune skin diseases discovered a 98% positive correlation between the two staining patterns. They advised routinely using PAS in conjunction with H&E, particularly in labs without immunofluorescence equipment. This result is similar to that of the current investigation, in which PAS staining consistently revealed patterns of floor and roof staining that corresponded to certain subepidermal bullous diseases and highlighted the basement membrane zone in all 40 instances. Furthermore, the prevalence of bullous pemphigoid (65%), universal subepidermal blister

formation, and eosinophil-rich infiltrate all lend credence to PAS's diagnostic utility as a trustworthy supplement to standard histopathology, especially in settings with limited resources where DIF might not be easily accessible. (9)

Direct immunofluorescence is still the gold standard, according to Gardner and Crawford (2018), who found that common histopathological features, such as PAS staining and inflammatory infiltrate patterns, demonstrated limited reliability in distinguishing between epidermolysis bullosa acquisita and bullous pemphigoid. In contrast, the current investigation showed a preponderance of bullous pemphigoid (65%) and eosinophil-rich infiltration (55%), as well as consistent PAS basement membrane staining with unique floor (80%) and roof patterns that correlate with certain subepidermal bullous diseases. Therefore, our results suggest PAS as a valuable auxiliary diagnostic tool in routine histopathological evaluation, particularly in settings with limited resources, even though DIF is definitive.(13)

In the study conducted by Sindhoor et al (2024), most bullous pemphigoid cases showed subepidermal blistering with eosinophil-rich bulla and predominant floor pattern PAS staining, along with confirmation by DIF. These findings are similar to the present study, which also demonstrated universal subepidermal blister formation, predominance of bullous pemphigoid (65%), eosinophil-rich infiltrate (55%), and predominant floor pattern PAS staining (80%). This concordance supports the role of PAS as a useful adjunct to routine histopathology in the evaluation of subepidermal bullous disorders. (14)

The American Society of Dermatopathology's AUC review emphasized PAS as a useful and frequently used auxiliary stain in standard dermatopathology practice. This is in line with the current investigation, which demonstrated that PAS was a dependable supplement to standard histology by highlighting the basement membrane zone in every instance and displaying unique floor (80%) and roof patterns that corresponded with particular subepidermal bullous illnesses. (15)

The study conducted by Ponmar, Madhurima, et al (2024) Bullous pemphigoid was the most often diagnosed condition in the aforementioned study. PAS staining revealed a floor pattern in BP and LPP and a roof pattern in SLE and PCT, confirming PAS's use as a supplement to H&E. To further support the utility of PAS in distinguishing subepidermal bullous illnesses, the current study also demonstrated a preponderance of bullous pemphigoid (65%), global subepidermal blistering, and predominant floor pattern PAS staining (80%). (16)

The most prevalent subepidermal vesiculobullous disorder, according to a study by Punnesetty et al., was bullous pemphigoid (62%), followed by erythema multiforme (26%). The condition was more common in women and peaked between the ages of 60 and 80. Additionally, they noted positive direct immunofluorescence (DIF) in 71.4% of patients and dermo-epidermal junction separation in all cases, highlighting the significance of clinicopathological correlation with DIF for precise subepidermal bullous disease characterisation. In the current investigation, bullous pemphigoid was the most prevalent entity (65%), with a female preponderance (57.5%) and ubiquitous subepidermal blister formation on histology. These results are essentially comparable. However, the current study concentrated on histomorphology and PAS staining patterns as supplementary diagnostic techniques, in contrast to their work, which heavily depended on DIF positive. (17)

With just 54% of cases exhibiting conventional subepidermal clefting, Hodge et al. brought attention to the pemphigoid's varied histological spectrum. However, the current investigation revealed that subepidermal blisters developed in every instance with a preponderance of eosinophil-rich infiltration. Additionally, PAS staining regularly revealed the basement membrane zone and showed clear diagnostic floor and roof patterns in our investigation, which helped to improve the distinction of subepidermal bullous diseases. (18)

Chen et al. found basal cell necrosis in 92.7% of bullous pemphigoid cases with strong correlation to eosinophil density. This is comparable to the present study, which also showed eosinophil-rich infiltrate and consistent subepidermal blistering in bullous pemphigoid. However, our study further emphasizes the diagnostic role of PAS staining in highlighting the basement membrane zone and differentiating subepidermal bullous disorders.(19)

According to Baigrie and Nookala, bullous pemphigoid is the most prevalent subepidermal blistering condition that primarily affects elderly people and is accompanied by subepidermal split and eosinophils. This is in line with the current investigation, which also revealed subepidermal blistering and an eosinophil-rich infiltrate with bullous pemphigoid as the major entity. Our study further emphasizes the supplementary function of PAS staining in diagnostic differentiation and basement membrane zone delineation.(20)

Conclusion

The most prevalent subepidermal bullous condition, bullous pemphigoid, was characterized by eosinophil-rich infiltration and persistent subepidermal blistering. The basement membrane zone was plainly visible thanks to PAS staining, which also revealed unique floor and roof patterns that correlated with particular diagnoses. These results show that PAS stain has a high diagnostic value when used in conjunction with standard H&E. Therefore, PAS is a straightforward, affordable, and trustworthy supplemental method for assessing subepidermal bullous lesions, particularly in environments with limited resources.

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