Original article:

Comparative microanatomy of the normal skin with that of immunobullous condition

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Abstract:

Introduction: Immunobullous diseases have been a topic of interest for long because of its morbidity and mortality.Among all immunobullous diseases Pemphigus Vulgaris, Pemphigus Foliaceus and Bullous Pemphigoid are common.

Methodology: The present study is directed to compare the microanatomy of normal skin with the skin affected by above mentioned three diseases.

Observations: For this 98 cases were taken randomly. Among them 35 were Pemphigus Vulgaris, 38 were Pemphigus Foliaceus and 25 were Bullous Pemphigoid. Biopsy was done.

Results: Great alteration of normal histology was observed after H & E staining and light microscopy.

Conclusion: Thus by comparing the pathological slides with the normal slides one can easily diagnose the diseases.

Key Words*:* Comparative microanatomy, Pemphigus Vulgaris

Introduction

Skin is the largest organ of our body covering the entire external surface. It is divided into two separate but functionally dependent layers - a cellular epidermis and a dermis [(1)] composed mainly of connective tissues. Pemphigus vulgaris and Pemphigus foliaceus are immunobullous diseases which occure due to the presence of autoantibodies against intracellular adhesive proteins (Desmoglein I or Desmoglein III) [(2)]. In Bullous pemphigoid the auto-antibodies are directed against two structural proteins (BPAG1, BPAG2) found in the hemidesmosomes of dermo-epidermal junctions [(3)]. Their primary features are bullae or blisters which occur due to loss of intercellular ahesions. These diseases are still a major health problem in our country. Histopathology is the gold standard for diagnosis till now.

In Pemphigus vulgaris, Pemphigus foliaceus and Bullous pemphigoid blisters occur at different level of epidermis. In Pemphigus foliaceus intra-epidermal bullae are formed [(4)], in Pemphigus vulgaris they are suprabasal [(5)] and in Bullous pemphigoid blisters occur subepidermally or at dermo-epidermal junction [(3)].For diagnostic purpose proper understanding of the normal histology of skin is very important since only then a deviation or an aberration, however minor it is will not be missed by the eye. The objective of the present study is to detect such immunobullous diseases by comparing the

histological features with the histology of normal skin seen under light microscope in the absence of sophisticated electron microscopy and immune-ofluroscence tests.

Materials & Methods

The study was conducted at the Department of Anatomy R G Kar Medical College. Detailed history along with punch biopsy was taken from every patient attending Dermatology OPD with bullous eruptions.

Patients with the following criteria were selected: having vesicles/ bullae, new untreated cases, with written consent and having no pregnancy. 98 patients were taken as subjects maintaining the above mentioned criteria. A control group was also taken with 50 persons without apparent skin diseases. A cross sectional study was done starting from March 2008 to July 2009. A fresh bulla preferably less than 24 hrs old was selected and the fluid was preferably cleared. Turbid fluid containing bulla was rejected. Fresh bulla was taken to prevent secondary changes such as regeneration, degeneration, scarring or secondary infection which may obscure essential features and make recognition of the primary pathologic process impossible. Punch biopsy was done as the standard procedure for collecting specimen. 4 mm biopsy punch was adequate. A 3 mm punch was preferred for small lesions or biopsies from the face or other areas where cosmesis was of concern. Biopsy from the corresponding areas as that of cases was also taken from control for studying normal skin histology.

After the skin specimen was loosened with the biopsy punch, it was handled very gently and above all, grasped with forceps at the very edge. A punch biopsy specimen was squeezed gently out of its socket or carefully spread with the syringe needle. The biopsy specimen was placed in fixative immediately after removal from the patient to prevent autolysis. It was not allowed to dry and was not allowed to touch the bottle. As a fixative 10% buffered formalin was used. Similar biopsy was also taken from persons having no bullae. Biopsy material was then stained with Haematoxylin and Eosin.

Observations

In Pemphigus vulgaris **(Table I, Figure 1)** epidermal thickness was reduced. There was presence of suprabasal cleft. Eosinophilic spongiosis and necrosis were present. Stratum basale had tombstone appearance. Upper epidermis was intact. Within suprabasal bullae there were presence of acantholytic cells, lymphocytes & eosinophil. In dermis there was presence of inflammatory cells -mainly lymphocytes. Neutrophils were present. In few cases there were vessel dilatation & perivascular infiltrtion. There were no infiltration & spongiosis around hair follicle & sweat apparatus. Subcutaneous tissue was normal.

In Pemphigus Foliaceus **(Table I, Figure 2)** subcorneal blisters were seen.The content of bullae was mainly neutrophil. There was occassional presence of eosinophil & acantholytic cells. Epidermal thickness was normal Spongiosis was present but necrosis was absent. (Roof necrosis may be present). As basal layer was intact there was no pigment incontinence. Inflammatory cells were present & they were mainly lymphocytes.Vessel dilatation and perivascular infiltration were absent. Hair follicle and sweat apparatus appeared to be normal. There was some infiltration around sebaceous glands. Subcutaneous tissue was normal.

In Bullous Pemphigoid **(Table I, Figure 3)** there were subepidermal blisters containing neut-rophil or eosinophil. Epidermal thickness was norm-

al. Spongiosis was absent. In few cases there was presence of roof necrosis. Stratum basale was intact.

There was presence of inflammatory cells mainly lymphocytes neutrophils & eosino-phils.Vessel dilatation and perivascular infiltration were present but pigment incontinence was absent.

Usually there was no infiltration & spongiosis around hair follicle & sweat apparatus. Subcutaneous tissue was normal.

Discussion

Pemphigus vulgaris is characterized by the suprabasal bullae. Trunk and extrimities are frequently involved which was also observed by Chandrashekhar et al.[6] In addition to skin leisons oral mucosa is also involved.This observation was also noticed by Fernandez et al.[7] In Pemphigus group of diseases the most important thing was acantholysis – epidermal cells loose their cohesiveness. These acantholytic cells can be demonstrated by cytological examination (Tzanck smear).

The early changes in Pemphigus Vulgaris consist of intracellular oedema with loss of intercellular attachments in the basal layer. Suprabasal epidermal cell separate from basal cells to form cleft and blisters [8]. The attachment of basal cells to the basement membrane is not affected. Eosinophilic spongiosis may be prominent with intraepidermal eosinophilic pustules.This spongiosis occurs due to loss of syndecan 1 expression which helps in intracellular adhesions and present on the keratinocytes. In spongiosis desmosomal stretching occurs prior to cell separation in contrary to acantholysis.[9] Regarding the histopathology findings of Pemphigus Foliaceus, blisters form in the intracellular spaces in the upper level of epidermis. These coalesce to form clefts and superficial bullae high in the granular layer or immediately below the stratum corneum. Eosinophilic spongiosis or neutrophilic spongiosis [10] may precede blisters.

Bullous pemphigoid usually affects elderly patients [11] and the incidence increases with age. In Bullous Pemphigoid the blister arises at dermo-epidermal junction [3].Epithelial migration and regeneration may be responsible for this. Biopsy of an early small blister is most diagnostic.In case of older lesions Direct Immunufluorecence (DIF) study should be done for diagnosis. With the help of light microscope it was seen that there was presence of scanty lymphocytic perivascular infiltration with few eosinophils mainly scattered throughout the dermis. Eosinophilic spongiosis may occur but it was absent among study cases. Other findings were similar to those reported by previous workers.

Conclusion

From this study it can be concluded that comparing the histology of normal skin with that of vesicobullous diseases, one can diagnose correctly the exact disease. But to get an idea about the agents responsible for such pathological lesions and the ultrastructural changes underwent by the diseased tissues, sophisticated techniques like immunofluorescense and electronmicroscopy are needed.

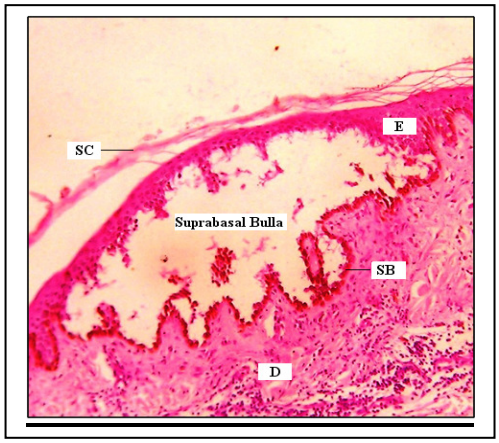

Figure 1 Pemphigus Vulgaris with Suprabasal Bulla (Bulla located in suprabasal layer): SC, *Stratum Corneum*; **E**, *Epidermis*; **SB**, *Stratum Basale*; **D**, *Dermis*

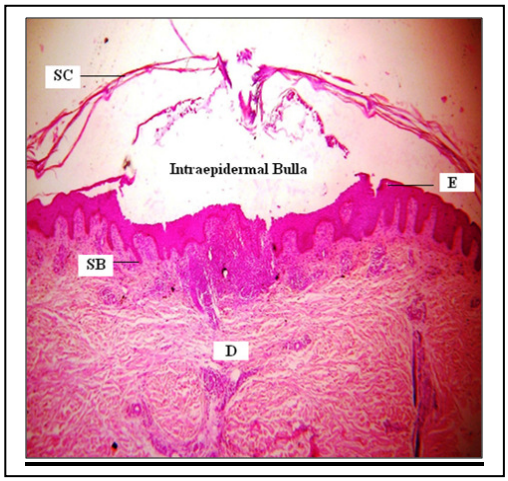

Figure 2 Pemphigus Foliaceus with Intraepidermal Bulla (Bulla located within epidermis): SC, *Stratum Corneum*; **E**, *Epidermis*; **SB**, *Stratum Basale*; **D**, *Dermis*

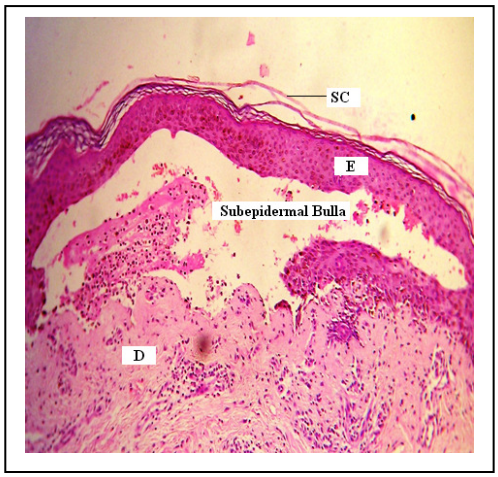

Figure 3 Bullous Pemphigoid with Subepidermal Bulla (Bulla located below the epidermis): **SC**, *Stratum Corneum*; **E**, *Epidermis*; **D**, *Dermis*

TABLES

Table I

Histological features studied

Layer of skin	Feature	Normal	Pemphigus Vulgaris	Pemphigus Foliaceus	Bullous Pemphigoid
Epidermis	Blister	Absent	Present - Suprabasal containing neutrophil, eosinophil, acantholytic cells, histiocytes	Present - Subcorneal containing neutrophil, eosinophil, acantholytic cells	Present - Subepidrmal containing neutrophil & eosinophil
	Thickness	100% normal	55% normal, 45% reduced	81% normal, 19% reduced	83% normal, 17% reduced
	Spongiosis	Absent	30% absent, 70% present	15% absent, 85% present	11% absent, 89% present
	Necrosis	Absent	25% absent, 75% present	19% absent, 81% present	83% absent, 17% present
	Stratum Basale	Intact	Intact, row of tombstone in 100%	Intact	Intact

Dermis	Inflammatory cells	Few & scattered	Mainly lymphocyte, neutrophil, eosinophil	Mainly lymphocyte, neutrophil, eosinophil	Mainly lymphocyte, neutrophil, eosinophil
	Vessel dilatation	Absent	80% present, 20% absent	77% present, 23% absent	82.4% present, 17.6% absent
	Perivascular infiltration	Lymphohistiocytic in few cases, scattered macrophages in 10% cases of normal skin	100% present	91% present, 9% absent	88.3% present, 11.7% absent
	Hair follicle	No infiltration	Apears to be normal	Apears to be normal	5.88% present, 94.12% absent
	Sweat apparatus	No change	80% no infiltration, 20% mild	No infiltration	No infiltration
Subcutaneous tissue		100% no change	100% normal	100% normal	100% normal

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