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A histomorphological study of bullous lesions of skin with special reference to immunofluorescence

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A B S T R A C T

Vesiculobullous eruptions are encountered in heterogenous groups of dermatoses. Definite diagnosis of vesiculobullous disorder requires histomorphological diagnosis along with Direct Immunofluorescence (DIF) and clinical findings. This study was undertaken to evaluate the utility of DIF in histopathological diagnosis. A total of 50 cases of vesiculobullous disorders were studied over a span of 24 months from 1st July 2011 to 30th July 2013. Total of 50 skin biopsies from patients with vesiculobullous skin lesions were sent to the Department of Pathology, B R Ambedkar Medical College, Bangalore. Punch biopsies were taken for histopathological diagnosis. H& E stain was applied. Perilesional skin was taken in normal saline for DIF procedure. In the present study pemphigus vulgaris constituted the most common vesiculobullous disorder (34%) followed by bullous pemphigoid in 26% of cases. Majority of patients presented between 40-49 yrs of age with female prepondarance. Almost all cases presented with blister. Suprabasal blister was seen in Pemphius Vulgaris along with acantholytic cells. Subcorneal blister was noted in Pemphigus foliaceus and Sub corneal pustular dermatosis. Dermoepidermal junction separation was seen in Bullous Pemphigoid, Dermatitis Herpitiformis, and Bullous SLE. DIF showed positive findings in 70% cases. Only 1 case showed HPE and DIF discordance. DIF is not a substitute but supplement to histopathological diagnosis. Thus a clincopathological correlation with DIF is required for definite diagnosis.

Introduction

Skin forms not only a protective covering but is a part of immune apparatus of body (Ranjana et al, 2010). Skin is the single largest organ of the body. Skin represents a window to the internal well-being of disease. Many internal diseases may manifest themselves in the skin (Wojnarowska et al, 2010).

Vesiculobullous disorders represent a heterogenous group of dermatoses with protean manifestations. They have dramatic

impact on the patient and their family and have severe economic consequences for the family and health services. The diseases have been the subject of intensive investigation in recent years (Wojnarowska et al, 2010).

There are a wide variety of bullous diseases, some of which can be extremely debilitating and even fatal, some bullous lesions may have serious sequele, necessating early treatment and intervention to prevent further morbidity and mortality (Tani et al, 1984). Clinical examination of skin bullous lesion provides dermatologist gross morphological finding upon which differential diagnosis can be found out. However histopathological examination is needed for definite diagnosis (Kabir, 2009). Bullous lesions are frequently a source of dismay to pathologist. Skin biopsie are easily intended with precision, direct immunofluorescent microscopy in conjugation with histopathology gives the best diagnostic yield in bullous lesions to make a clear reporting (Ranjana et al, 2010).

Bullous lesions can be classified based on site, shape and size of the bulla and also changes in the bulla, epidermis and dermis (Gane, 1973). Blisters in the various disorders occur at different levels within the skin. Histologic assessment is essential for accurate diagnosis and provides insight into the pathogenic mechanisms. Knowledge of the molecular structure of the intercellular and cell-to-matrix attachments that provide the skin with mechanical stability is helpful in understanding this diseases (Lazar et al, 2010).

Diagnosis of disease requires thorough histopathological examination. Initial basis of identification starts with the site of lesion followed by classification according to location as suprabasal, intraepidermal, sub corneal and sub epidermal group, then change within the bullous lesion is seen that is presence of acantholytic cells and inflammatory cells. Adjacent epidermal changes are also noted like villi, accentuation of normal dermal papillae, hyperkeratosis, parakeratosis, spongiosis and acanthosis and also site of disease and age of patient is important in diagnosis (Gane, 1973).

Only H&E in bullous lesion does not yield much result. Bullous lesion are immune mediated and immunopathogensis patterns are disease specific and are of diagnostic importance, many of this bullous lesion show immune perturbation as a part of disease pathogenesis at various location such as dermo-epidermal junction, dermal blood vessels etc. Nature of immune deposits usually used in DIF is IgG, IgA, IgM and C3 (Abresman et al, 2001).

Immunofluorescence techniques are essential to supplement clinical findings and histopathology in the diagnosis of the immunobullous disorders. These rapid and reliable techniques permit early diagnosis and treatment of potentially life-threatening disorders.

By Direct Fluorescent Microscopy presence of immunocomplex can be detected and will help to arrive at diagnosis. DIF is considered diagnostic tool in detection of mostly subepidermal autoimmune diseases (Tariq et al, 2003).

Recent advances in investigative dermatology have created new horizons. Over the last two decades, great advances have been made in understanding the clinical behavior and molecular nature of autoimmune diseases (Lazar et al, 2010).

Hence this study was done to study the histopathological changes of skin in

vesiculobullous lesions, to study bullous lesions in all aspects using haematoxylin eosin stain and immunofluorescence, to study the most common condition associated with bullous lesions, to provide vital data for subsequent treatment regimen and to record the sensitivity of immunofluorescence in vesiculobullous lesions.

Materials and Methods

This study was conducted in pathology department of Dr B R Ambedkar Medical college & hospital in collaboration with Department of dermatology. Minimum of 50 cases of bullous lesion of skin was collected from June 2011 to July 2013 in the present study. These patients had clinical history of bullous lesions. Biopsy was fixed in 10% formalin and PBS. Histological slides were prepared and studied using H&E stain and immunofluorescence.

Result and Discussion

The present study was conducted over a period of 24 months from 1st July 2011 to 30th July 2013 in the department of Pathology, at Dr B R Ambedkar Medical College, Bangalore. The results were as follows:

In the present study pemphigus vulgaris constituted the most common vesiculobullous disorders constituting 34% [17 out of 50 cases] followed by Bullous pemphigoid 26% [13out of 50 cases] Pemphigus foliaceus , Subcorneal pustular dermatosis, Songiotic dermatitis constituted 8%[4 out of 50 cases]. Least common was bullous SLE which constituted 2% [1 out of 50 cases].

In present study majority of patients presented between the age group of 40-49 yrs (24 %). Youngest patient in the study was 9 years old and the oldest being 82 years. Comparatively Females outnumbered the males in this present study. Male to female ratio was 1:1.5.

In present study Pemphigus vulgaris presented most commonly in age group of 30-39 years [35.3%] followed by 40-49 [29.45] years age group. Bullous pemphigoid presented commoly in the age group of 70-79 years [38.4%] . Pemphigu foliaceus and BSLE were common at age group of 20-29 years [50% and 100%].

In Present study PV, PF, EM and BP showed predominantly female predominance [70.%, 75%, 66.6% and 53.8% respectively]. EM, BDE showed male predominance [100%].

In this study it was noticed that 42 cases [84%] presented with blisters. PV showed blisters in 94.2% of cases, BP showed blisters in 100% of cases. SCPD and EM less commonly presented with blisters.

In 12 out of 17 cases (71%) of PV burning sensation was the chief complaint followed by pain (17.6%) and itching (11.8%). Itching was the most common symptoms in BP and was seen in 11 out of 13cases (84.6%). 75% of SD cases did not present with any symptoms.

In this study vesicle/bulla was the common primary lesion. PV, BP, PF DH, BDE and BSLE mainly presented as bulla. SCPD mainly presented as pustule.

60% of cases had erythematous base. PV, BP had erythematous base in 70.5% and 69.3% cases respectively. PF, SCPD, DH, BSLE also showed erythematous base in most of its cases. Nikolskys sign was present in 30 out of 50 cases [60%] most common in

PV and PF with 88.2% and 75% respectively. BSS was negative in 76% of cases.

In present study it was noticed that 100% case of PF, 52.9% of PV, 38.5% of BP, 75% of SCPD, 66.6% of EM showed crusts. Erosion was noticed in 58.8% of PV, 53.8% of BP, 50% of SCPD and BDE. Pigmentation was noted in PF, BSLE, SD and BP. Vegetation was noted in any condition.

Oral mucosa involvement was present in 100% cases of DH and BSLE .It was seen in 88.2% cases of PV. Only 50% involvement was seen in SCPD and SD.

Most common presentation of cutaneous lesion was all over the body in vesiculobullous lesion; Predominant in PV [70.5%] followed by BP [69.2%]. Next common presentation was over the trunk and face in PV. Limb involvement was seen in BP [7.6%] and EM [33.3%].

In present study 88.2% of PV and 100% of PF showed suprabasal separation. Dermo-epidermal junction separation was seen in 92.3% cases of BP, 66.6% of EM, 100% of DH, BDE and BSLE respectively. 100% cases of SD showed intraepidermal separation. Remaining 5.8% of PV 7.6% of BP, 33.3% of EM did not show any separation

Tomb stone appearance [70.5%] and villi [17.6%] was noted only in PV. Hyperkertosis was seen in 29.4% of PV, 25% of EM, 50% of BDE, 100% BSLE. Acanthosis was noted in 100% of PF, SD and 94% of PV. Dyskeratosis was noted in 100% of PF 25% of SD and EM. Acanthocytes was predominantly in PV [94.5%], PF [.100%], SD [75%], SPCD[50]. Apototic cells were noted only in SD.

PV showed 88.2% of dermal infiltration and 47% perivascular infiltration. BP showed 84.6% of dermal infiltration and 47% of perivascular infiltration. PF, SCPD, SD, EM, DH, BDE, BSLE also showed dermal and perivasular infiltration. Dermal edema was noted in BP [7.6%], EM [33.3%], DH [50%]. Adenxal infiltration was noted in BP [7.6%] and PF [25%]

PV, PF SCPD, SD, DH and BSLE predominantly showed neutrophils. Eosinophils was seen in Bullous pemphigoid and BDE. Mixed inflammation was seen in PV and BP DIF was positive in 70% and negative in 20% of cases.

IgG was predominatly positive in PV (52.5%), PF (75%), DH (50%). C3 was seen in BP (46.15%). Both IgG and C3 was positive in PV (41.17%), BP (46.15%). DIF was negative in EM, BDE. DIF was not done in 2 out of 4 cases in SCPD and SD and 2 showed negative results. IgA along with IgG was positive in DH and BDE

In all positive cases of PV, PFshowed 100% deposition of antibodies in squamous intracellular spaces. Dermoepidermal junction deposition of antibodies was noted in 100% cases of BP and BSLE. Only one case of pemphigus vulgaris showed discordance with DIF.

The present study was conducted over a period of 24 months from 1st July 2011 to 30th July 2013 in the Department of Pathology, at Dr B R Ambedkar Medical College, Bangalore. In the present study clinical, histopathological and direct immunofloresence of various vesiculobullous diseases have been discussed and compared with various other studies in detail below.

It was an observational study with short study case; no statistical tests were applied for the analysis of data and the results were expressed in numbers and percentages. As it was a hospital-based study, the above number does not reflect the true incidence of vesiculobullous disorders in the community.

In the present study pemphigus vulgaris was the most common vesiculobullous disorder constituting 34% (17 out of 50 cases) followed by bullous pemphigoid 26%(11 cases). PF being 8 % (4 cases) and EM being 6%. This study showed similar results as that of Inchara YK et al (2007) study. Present study also included SCPD, DH, SD, and BDE. PV being the most common is similar to Tsankov N et al. (2000) , Nanda A et al (2004) , Nurul Kabir AKM et al (2008) studies. The present study showed various vesiculobullous disorder like SCPD, DH and BSLE which are not seen in other studies.

In present study bullous pemphigoid most common age was above 70 years similar to Bertram F et al. (2009) , Uzun s et al (2006), Lagan SM et al (1978), Joly P et al (2012) studies. PV ranged from 30-49 yrs similar to Lagan SM et al (1978) study. DH did not show similarity with Bertram F at el (2009) study.

In the present study, pemphigus vulgaris constituted 17 of the total number of cases of vesiculobullous disorders, which is lower than that of Kanwar AJ et al (2011) , Vora D et al (2010) , Nafiseh I et al (2007) study. This shows that the disease has geographic changes. Female to male ratio in this study was 1:2.4 similar to study Nafiseh et al (2007) , Vora D et al (2010) , and Kanwar et al (2011) study. Mucosal involvement was seen in 88.2% in present study similar to other studies. Nikolskys sign was positive in 88.2% cases similar to Vora D et al (2010) study. Symptoms prominent in this study was burning and itching and showed a generalized distribution pattern similar to Vora D et al (2010) study.

Suprabasal bulla was seen in 88.2% same as that of Arya SR et al (1999) study. Acantholysis was seen in 16 cases (94%) which is same as that observed by Arya SR et al (1999) . Vora D et al (2010) study.

Row of tombstone appearance seen in 12 (70.5%) of cases which is higher than the Arya SR et al (1999) and Vora D et al (2010) study. Inflammatory cells were noted in 94% which is again higher than that of all three above mentioned study.

Direct immunofluorescence was done in all 17 cases of pemphigus vulgaris. 16(94.11%) cases were positive, 1 was negative. As compared to Kaur JS et al (1992) study which showed 100 % positive DIF. Chams-Davatchi C et al (2005) study had 417 cases of pemphigus vulgaris out of 1111, among which 389 (93.28%) were positive. This shows that even the most definitive investigation may be negative. So the diagnosis depends on clinical, histopathological and immunofluorescence study.

Age group was mainly between 20-40 years in this study with M: F ratio 1:3 which was opposite to that of Vora D et al (2010) study. All lesions presented as vesicle with 1 case also presenting as papule. Mucosal membrane involvement noted in 25% cases which is similar to Arya SR et al (1999) study.

Histopathology: Nikolskys sign showed positivity in 75% cases which is lower than Arya et al (1999) study. Subcorneal bulla and acantholysis showed 100% positivity similar to Vora D et al (2010) study. Inflammatory cell was seen in 75% cases

which did not show similarity with above study.

Direct immunofluorescence was done in all cases of pemphigus foliaceus and the findings were suggestive of pemphigus foliaceus in all the cases (100%). Chams-Davatchi C et al (2005) study showed 88 %. DIF positive and Inchara YK et al (2007) showed 100 % DIF positive. DIF finding are similar to PV but histopathology differentiates between PV and PF. So DIF is just supplement but not a substitute

Present study had 4 cases within age group of 40- 50 years which is lower then Lutz ME et al (1998) study. 3 cases presented with pustule and crust. M/F ratio showed equal distribution between male and female.

Histopathology mainly showed sub corneal blister containing neutrophils as inflammatory cells. DIF was not done in 2 cases, negative in 2 cases where as Lutz ME et al (1998) showed intercellular spaces deposit of IgA in 3 cases.

In present study bullous pemphigid constituted 26% with mean age of the patient in the range of 40-79 years. Male to female ratio (M: F) ratio being 1:1.1 which is similar to Lagan SM et al (2008) and Budimir J et al (2008) study. All patient of bullous pemphigoid presented with bulla. Itching (84.6%) was the chief complaint in all patient. 69.3% presented with erythematous base. 53.8% showed positivity for Nikolsky sign. Only 23% of patient showed oral involvement

11 cases out of 13 (76.4%) showed sub epidermal blister. One case had suprabasal cleft. This might be due to an older lesion being biopsied. Inflammatory cells were noted in bulla (92.3%) and dermal infiltrate (84.7%) similar to Leena JB et al (2012) study. Predominant inflammatory cells were eosinophils similar to Nishioka et al (1984) study.

In present study DIF was done in 12 cases. DIF was not done in 1 case because of delay in sample collection. All 12 cases showed 100% positivity similar to Deepthi PK et al (2013) , Cozzani E et al (2001) study.

Dermatitis herpitiformis

2 cases presented with DH which constituted 8%. Cases were in the age group of 10-19 and 20-29 years respectively. Both of them presented as pustules. In one bulla was also noted; Subepidermal bulla was present in both cases with both showing papillary microabscess. DIF was positive in 2 of the cases showing granular deposit of IgA in dermoepidermal junction similar to Banu L et al (2012) study.

Bullous systemic lupus erythematosis

One female patient aged 24 years presented with bulla and pigmentation with face involvement. Positive Nikolsky sign and erythematus base was noted. This study had similar findings to that of Chan LS - 1999 et al (1999) study of a 15 year old female.

HPE: Showing subepidermal blister with neutrophil infiltration in blister cavity and dermis. DIF showed linear deposition of IgG and C3 along dermo- epidermal junction. Present study had 3 patients in peadiartic age group same as Mateos M et al (1998) study. 2 out of 3 patients were females. Only1 patient presented with bulla. One patient had pigmentation similar to Mateos M et al (1998) study.

HPE: 2 cases showed subepidermal blister with inflammatory cell predominantly lymphocytes and eosinophils. 1 case did not

show any separation. DIF was negative in all 3 cases.

Bullous drug eruption

2 cases with BDE where noted in present study in the age group of 30-49 years little lower than Cheng-Han L et al (2012) study. Both cases were male patient similar to Cheng –Han L et al (2012) study. Blister was seen in both patients with mucosal involvement in 50%. Cheng-Han L et al (2012) study showed mucosal involvement in 66.7%. Histopathology showed bulla in dermoepidermal junction, perivascular infiltration was predominant. Blister showed predominantly eosinophilic infiltration similar to Chen- Han L et al (2012) study. DIF in both cases showed negativity.

Table.1 Distribution of cases

Type	Frequency	Percentage
PV	17	34%
BP	13	26%
PF	4	8%
SCPD	4	8%
SD	4	8%
EM	3	6%
DH	2	4%
BDE	2	4%
BSLE	1	2%

Table.2 Age and sex distribution

Age (in years)	Male	Female	Total	Percentage
0 – 9	1	0	1	2%
10-19	2	1	3	6%
20-29	2	6	8	16%
30-39	4	7	11	22%
40-49	4	8	12	24%
50-59	0	1	1	2%
60-69	2	4	6	12%
70-79	4	3	7	14%
80 and above	1	0	1	2%
Total	20	30	50	100%
Percent %	40	60	100	

Table.3 Age distribution of vesiculobullous disorders

FD	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80 & >
PV	-	-	4(23.5%)	6(35.3%)	5(29.4)	1(5.8%)	-	1(5.8%)	-
BP	-	-	-	1(7.6%)	2(15.3%)	-	4(30.7%)	5(38.4%)	1(7.6%)
PF	-	1(25%)	2(50%)	1(25%)	-	-	-	-	-
SCPD	-	-	-	-	4(100%)	-	-	-	-
SD	-	-	-	1(25%)	1(25%0	-	1(25%)	1(25%)	-
EM	1(33.3%)	1(33.3%)	-	1(33.3%)	-	-	-	-	-
DH	-	1(50%)	1(50%)	-	-	-	-	-	-
BDE	-	-	-	1(50%)	1(50%)	-	-	-	-
BSLE	-	-	1(100%)	-	-	-	-	-	-

Table.4 Sex disrtibution of vesiculobullous disorders

FD	**Male**	**Female**
PV	5(29.4%)	12(70.6%)
BP	6(46.2%)	7(53.8%)
PF	1(25%)	3(75%)
SCPD	2(50%)	2(50%)
SD	2(50%)	2(50%)
EM	1(33.3%)	2(66.6%))
DH	1(50%)	1(50%)
BDE	2(100%)	-
BSLE	-	1(100%)

Table.5 Blisters in vesiculobullous disorders

FD	**Present**	**Absent**
PV	16 (94.2%)	1(5.8%)
BP	13(100%)	-
PF	3(75%)	1(25%)
SCPD	1(25%)	3(75%)
SD	2(50%)	2(50%)
EM	1(33.3%)	2(66.6%)
DH	2(100%)	-
BDE	2(100%)	-
BSLE	1(100%)	-
Total	42(84%)	8(16%)

Table.6 Associated symptoms in vesiculobullous disorders

FD	No symptom	Burning	Itching	Photosensitivity	Pain
PV	-	12(71%)	2(11.8%)	-	3(17.6%)
BP	-	2(15.4%)	11(84.6%)	-	-
PF	-	2(50%)	2(50%)	-	-
SCPD	-	-	1(25%)	-	3(75%)
SD	3(75%)	-	1(25%)	-	-
EM	-	1(33.3%)	1(33.3%)	-	1(33.3%)
DH	-	1(50%)	-	1(50%)	-
BDE	1(50%)	-	1(50%)	-	-
BSLE	-	1(100%)	-	-	-

Table.7 Morphology of primary lesion

FD	Papule	Vesicle/Bulla	Pustule
PV	0	16(94.2%)	1(5.8%)
BP	0	13(100%)	0
PF	1(25%)	3(75%)	0
SCPD	0	1(25%)	3(75%)
SD	2(50%)	2(50%)	0
EM	1(33.3%)	1(33.3%)	1(33.3%)
DH	0	1 (50%)	2(100%)
BDE	0	2(100%)	0
BSLE	0	1(100%)	0

Table.8 Morphology of vesiculobullous disorders

FD	Base		Nikolsky`s sign		Bulla spread sign	
	Erythmatous	Non-Erythematous	Present	Absent	Present	Absent
PV	12(70.5%)	5(29.5%)	15(88.2%)	2(11.8%)	6(35.3%)	11(64.7%)
BP	9(69.3%)	4(30.7%)	7(53.8%)	6(46.2%)	4(30%)	9(69.2%)
PF	3(75%)	1(25%)	3(75%)	1(25%)	1(25%)	3(75%)
SCPD	2(50%)	2(50%)	2(50%)	2(50%)	1(25%)	3(75%)
SD	1(25%)	3(75%)	1(25%)	3(75%)	0	4(100%)
EM	0	3(100%)	0	3(100%)	0	3(100%)
DH	2(100%)	0	0	2(100%)	0	2(100%)
BDE	0	2(100%)	1(50%)	1(50%)	0	2(100%)
BSLE	1(100%)	0	1(100%)	0	0	1(100%)
Total	30(60%)	20(40%)	30(60%)	20(40%)	12(24%)	38(76%)

Table.9 Morphology of secondary lesion

FD	Crust	Erosion	Pigmentation
PV	9(52.9%)	10(58.8%)	0
BP	5(38.5%)	7(53.8%)	2(11.8%)
PF	4(100%)	1(25%)	2(50%)
SCPD	3(75%)	2(50%)	0
SD	1(25%)	1(25%)	1(25%)
EM	2(66.6%)	1(33.3%)	0
DH	1(50%)	0	0
BDE	1(50%)	1(50%)	0
BSLE	0	0	1(100%)

Table.10 Oral mucosa involvement

FD	Present	Percentage %
PV	15	88.2
BP	03	23
PF	01	25
SCPD	02	50
SD	02	50
EM	03	100
DH	01	50
BDE	01	50
BSLE	01	100

Table.11 Cutaneous lesion of vesiculobullous lesions

FD	Absent	All over	Scalp	Trunk	Nail	Eyes	Limb	Face	Groin
PV	1(5.8%)	12(70.5%)	0	3(17.6%)	0	0	0	1(5.8%)	0
BP	0	9(69.2%)	0	2(15.3%)	0	0	1(7.6%)	0	0
PF	0	1(25%)	0	2(50%)	0	0	0	1(25%)	0
SCPD	1(25%)	2(50%)	0	0	0	0	0	1(25%)	0
SD	0	2(50%)	0	2(50%)	0	0	0	0	0
EM	0	2(66.6%)	0	0	0	0	1(33.3%)	0	0
DH	0	1(50%)	0	0	0	0	0	1(50%)	0
BDE	1(50%)	1(50%)	0	0	0	0	0	0	0
BSLE	0	0	0	0	0	0	0	1(100%)	0

Table.12 Level of blister

FD	No seperation	Suprabasal	Subcorneal	D:E Junction	Intraepidermal
PV	1(5.8%)	15(88.2%)	0	0	1(5.8%)
BP	1(7.6%)	0	0	12(92.3%)	0
PF	0	0	0	0	0
SCPD	0	0	4(100%)	0	0
SD	0	0	0	0	4(100%)
EM	1(33.3%)	0	0	2(66.6%)	0
DH	0	0	0	2(100%)	0
BDE	0	0	0	2(100%)	0
BSLE	0	0	0	1(100%)	0

Table.13 Epidermal changes

FD	**Tomb stone appearance**	**Villi**	**Hyper keratosis**	**Acanthosis**	**Dyskeratosis**	**Acanthocytes**	**Apoptotic cells**
PV	12(70.5%)	3(17.6%)	5(29.4%)	16(94%)	0	16(94%)	0
BP	0	0	0	0	0	0	0
PF	0	0	0	4(100%)	4(100%)	4(100%)	0
SCPD	0	0	0	1(25%)	0	2(50%)	0
SD	0	0	0	4(100%)	1(25%0	1(75%)	2(50%)
EM	0	0	1 (25%)	0	1(25%)	0	0
DH	0	0	0	0	0	0	0
BDE	0	0	1(50%)	1(50%)	0	0	0
BSLE	0	0	1(100%)	0	0	0	0

Table.14 Dermal changes

FD	**Dermal edema**	**Papillary microabseces**	**Melanin incontinence**	**Dermal inflitration**	**Perivarscular infiltration**	**Adenxal infiltration**
PV	0	0	0	15(88.2%)	8(47%)	0
BP	1(7.6%)	0	0	11(84.6%)	6(46.15%)	1(7.6%)
PF	0	0	0	4(100%)	3(75%)	1(25%)
SCPD	0	0	0	3(75%)	3(75%)	0
SD	0	0	0	4(100%)	1(75%)	0
EM	1(33.3%)	0	0	1(33.3%)	1(33.35)	0
DH	1(50%)	2(100%)	0	2(100%)	2(100%)	0
BDE	0	0	0	2(100%)	2(100%)	0
BSLE	0	0	0	1(100%)	1(100%)	0

Table.15 Inflammatory cells in blister

FD	Absent	Neutrophil	Lymphocyte	Eosinophil	Macrophage	Mixed
PV	1(5.8%)	10(58.8%)	0	0	0	6(35.2%)
BP	1(7.6%)	0	0	10(76.4%)	0	2(15.3%)
PF	1(25%)	2(50%)	1(25%)	0	0	0
SCPD	1(25%)	3(75%)	0	0	0	0
SD	0	3(75%)	0	0	0	1(25%)
EM	1(33.3%)	0	1(33.3%)	1(33.3%)	0	0
DH	0	2(100%)	0	0	0	0
BDE	0	0	0	2(100%)	0	0
BSLE	0	1(100%)	0	0	0	0

Table.16 Direct immunofloresence results

DIF	Frequency	Percentage
Not done	5	10%
Positive	35	70%
Negative	10	20%

Table.17 Antibody deposition

FD	IgG	IgM	IgA	C3	negative	Not done	IgG+c3	IgG+IgA
PV	9(52.95)	0	0	0	1(5.8%)	0	7(41.17%)	0
BP	0	0	0	6(46.15%)	0	1	6(46.15%)	0
PF	3(75%)	0	0	0	0	0	1(25%)	0
SCPD	0	0	0	0	2(50%)	2(50%)	0	0
SD	0	0	0	0	2(50%)	2(50%)	0	0
EM	0	0	0	0	3(100%)	0	0	0
DH	1(50%)	0	0	0	0	0	0	2(100%)
BDE	0	0	0	0	2(100%)	0	0	0
BSLE	0	0	0	0	0	0	1	1(100%)

Table.18 Pattern of deposition of antibodies in positive cases

FD	Squamous intercellular spaces	Dermoepidermal junction
PV	16(100%)	-
BP	-	12(100%)
PF	4(100%)	-
SCPD	-	-
SD	-	-
EM	-	-
DH	-	2(100%)
BDE	-	-
BSLE	-	1(100%)

Table.19 Discordance of hpe with DIF

HD	DIF
PV	Negative

Table.20 Analysis of types of vesiculobullous disorders

No	Studies	PV	BP	PF	SCPD	SD	EM	DH	BDE	BSLE
1	Vora D et al, 2010	96%		4						
2	Tsankov N et al, 2000	77.03		17.57						
3	Nanda et al 2004[11]	48	27							
4	Nurul kabir AKM et al 2008	23.5	22	20.6						
5	Inchara YK et al, 2007	29	22	7			3			
6	Present study	34%	26%	8%	8%	8%	6%	4%	4%	2%

Table.21 Age distribution pattern of the bullous lesions of the present study in comparison with other studies

Lesion	Bertram F et al.	Uzun S et al.	Lagan M Set al.	Joly P et al.	Present study
Bullous pemphigoid	70-80yrs	49-89	21-102yrs	49-106	60-80
Pemphigus vulgaris	60-70	18-70yrs	23-102	-	20-49
Pemphigus foliaceus	-	30-70yrs		-	20-40.
Dematitis herpitformis	40-50	-		-	10-29

Table.22 Clinical features comparsion

Features	Nafiseh Iet al	Kanwar AJ et al. study	Vora D et al	Present study
Years	2013	2011	2010	2011
No of cases	140	71	72	17
M:F ratio	1:1.59	1:1	1:1.485	1:2.4
Mucosal membrane involvement	77.5%	53.2%	100%	88.2%
Nikosky sign	-	-	87.90%	88.2%

Table.23 Comparison of HPE features

No	Features	Arya SR et al.	Handa et al.	Vora D et.	Present study (n=17)
1	Suprabasal bulla	81.4	31	54.16%	15(88.2%)
2	Acantholysis	93	31	91.6%	16(94%)
3	Row of tombstone appearance	41.8	-	55.5%	12(70.5%)
4	Inflammatory infiltrate in the bullous cavity	53.5	31	56.9%	16(94%)

Table.24 DIF findings in PV

PV	No of pt	DIF done	DIF positive
Present study	17	17	16(94.11%)
Chams-Davatchi C et al	1111	417	389(93.2%)
Kaur J S et al	20	10	10(100%)
Inchara YK et.al	29	29	26(89.7)

Table.25 Clinical findings and HPE

Features	Vora D et al	Arya SR et al	Present study
No of cases	3	25(35.7%)	4
M:F ratio	3:1	-	1:3
Mucosal membrane involvement		20%	25%
Nikolsky sign	1(33.3%)	94.7%	3(75%)
Subcorneal bulla	03(100%)	605	4(100%)
acantholysis	03(100%)	96%	4(100%)
Inflammatory cells	-	12(48%)	3(75%)

Table.26 DIF Findings in PF

PF	No of pt	DIF done	DIF positive
Chams-Davatchi C et al	89	34	30(88.2%)
Inchara YK et.al	7	7	7(100%)
Present study	4	4	4(100%)

Table.27 Clinical findings

SCPD	Lutz ME et al	Present study
No of cases	10	4
Age	66	40-50
pustules	10(100%)	3(75%)

Table.28 Clinical findings

Study	No of cases	Mean age	M:F ratio
Langan SM et al.	869	21-102yrs	1:1.5
Budimir J et al	18	70-95	1:1
Nanda A et al	28(27%)	65.97	1:5.75
Present study	13(26%)	40-79	1:1.1

Table.29 Histopathological Features of BP

No	**Present study**	**Leena JB et al**	**Nishioka K et al study**
Subepidermal blister	12(92.3 %%)	16(100%)	100%
Bulla content	12(92.3%)	16(100%)	-
Dermal infiltrate	11(84.7%)	16(100%)	10(40%)

Table.30 DIF Findings in BP

No	**Studies**	**No of cases**	**DIF done**	**DIF positive**
1	Cozzani E et al 2000[29]	32(100%)	32(100%)	32(100%)
2	Deepthi PK 2013[30]	8(13.3%)	8(100/%)	7(87%)
3	Present study	13(26%)	12(92.3%)	12(100%)

Table.31 HPE

DH	**Banu L et al 2012**	**Present study**
Cases	3(5.6%)	2(4%)
Subepidermal bulla	3(100%)	2(100%)
Papillary microabscess	3(100%)	2(100%)
DIF	3(100%)	2(100%)

Table.32 Clinical features

EM	**Mateos M et al 1998**	**Present study**
Cases	20	3
M:F	4:1	1:2
Age	Pediatric	Pediatric

Fig.1 Tense bulla noted in Pemphigus vulgaris

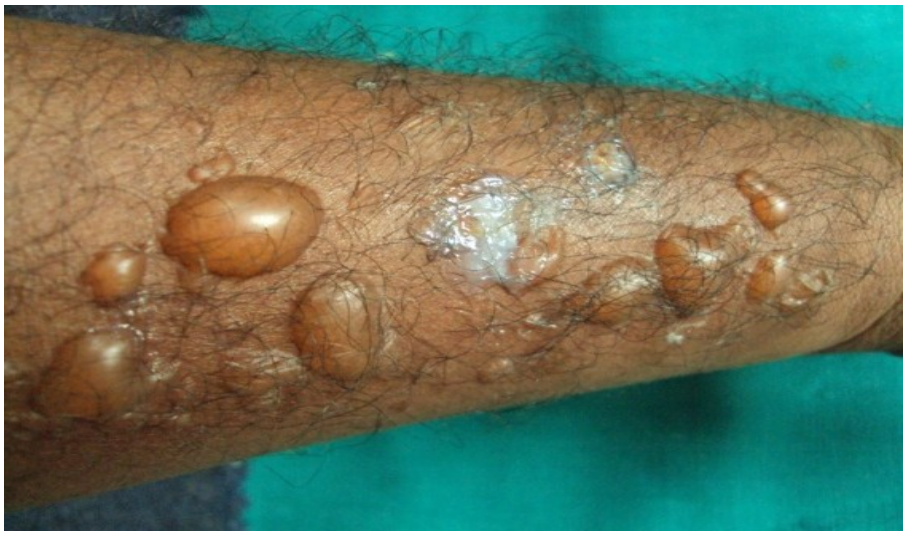


Fig.2 Tomb stone appearance with acanthocytes in blister cavity in PV (H&E, 10X)

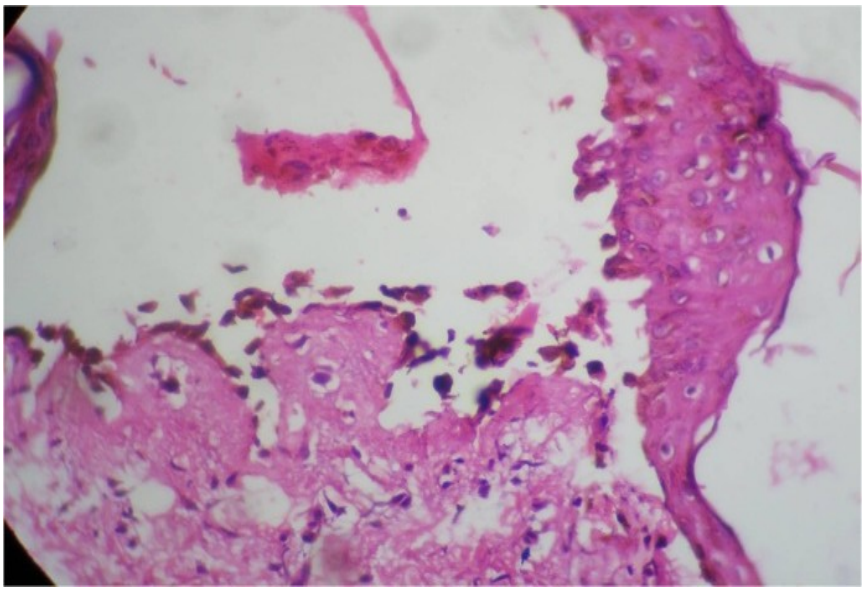


Fig.3 Supra basal bulla in PV (H&E, 10X)

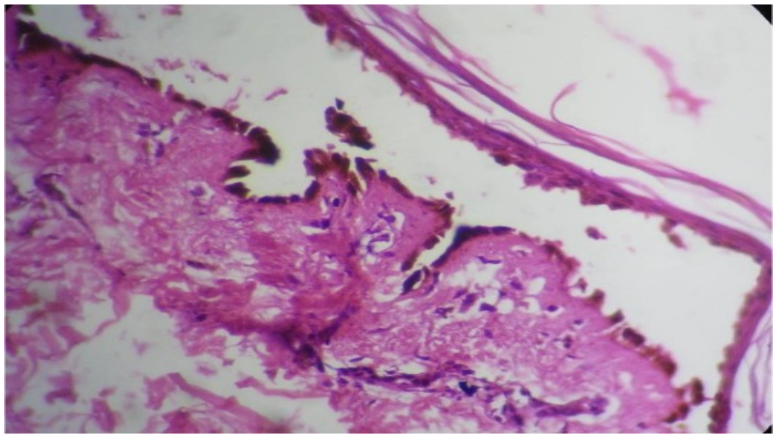


Fig.4 PV-Intercellular deposition of IgG (DIF, 10X)

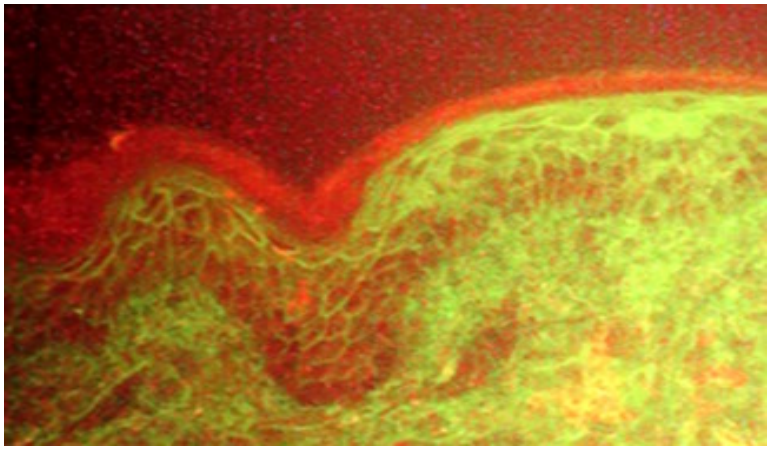


Fig.5 Small vesicles noted in Bullous Pemphigoid

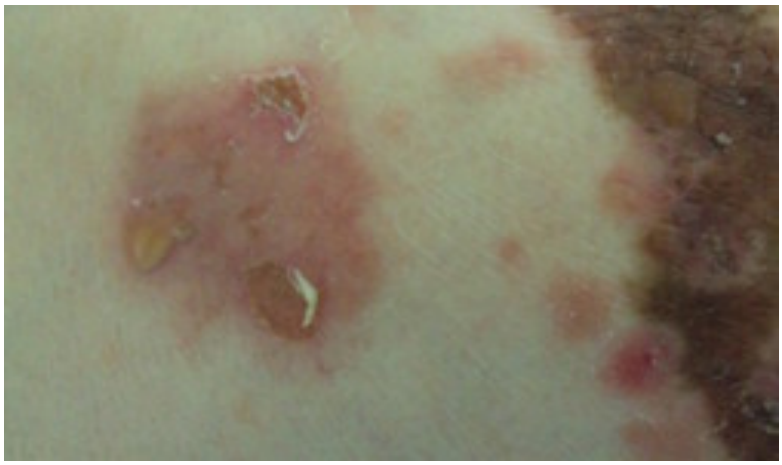


Fig.6 Separation at dermoepidermal junction in Bullous pemphigoid (H&E, 10X)

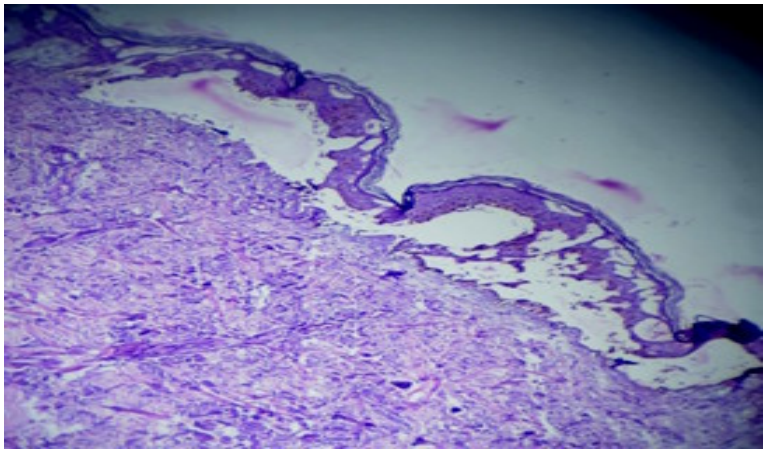


Fig.7 Cell poor blister cavity in Bullous pemphigoid (H&E, 10X)

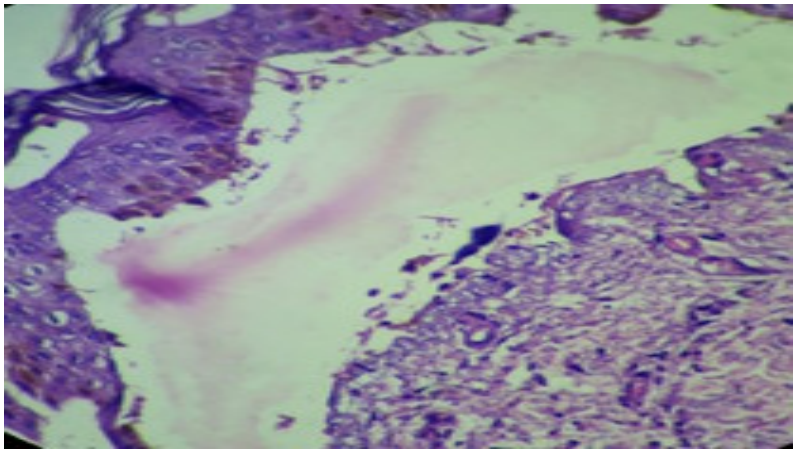


Fig.8 Linear deposition of C3 at Dermoepidermal junction in Bullous pemphigoid (DIF, 10X)

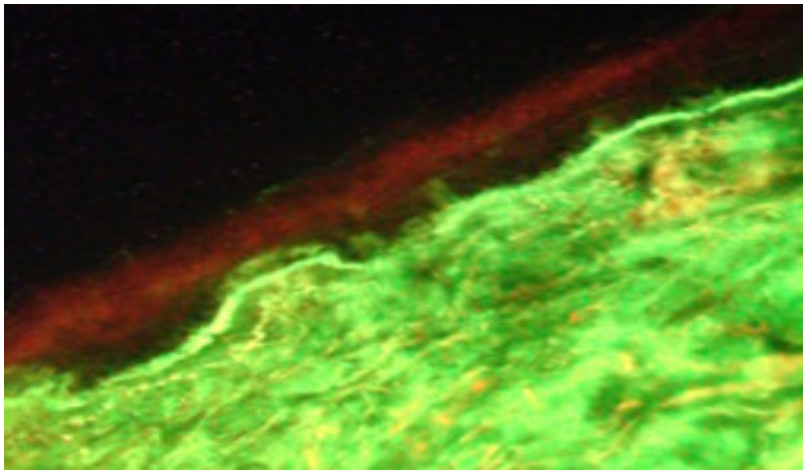


Fig.9 Tense blister with erosion in Pemphigus foliaceous

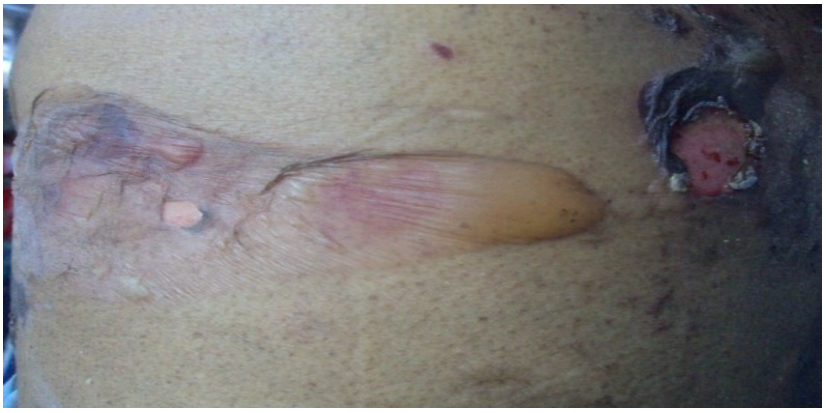


Fig.10 Subcorneal bulla with acanthocytes in blister cavity in Pemphigus foliaceous (H&E, 10X)

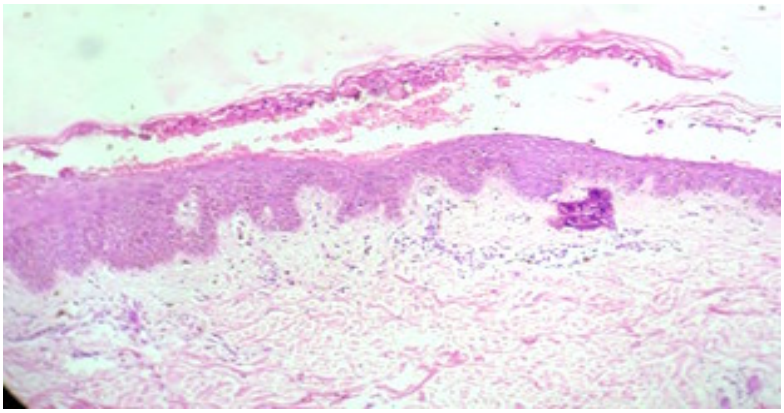


Fig.11 Intercellular deposition of C3 showing positivity in Pemphigus foliaceous (DIF, 10X)

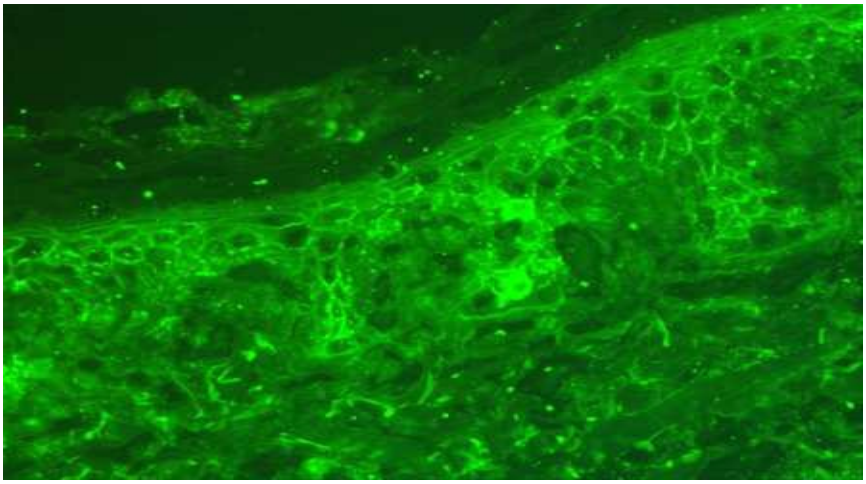


Fig.12 Subcorneal blister with inflammatory cells predominantly neutrophils in Subcorneal pustular dermatosis (H&E, 10X)

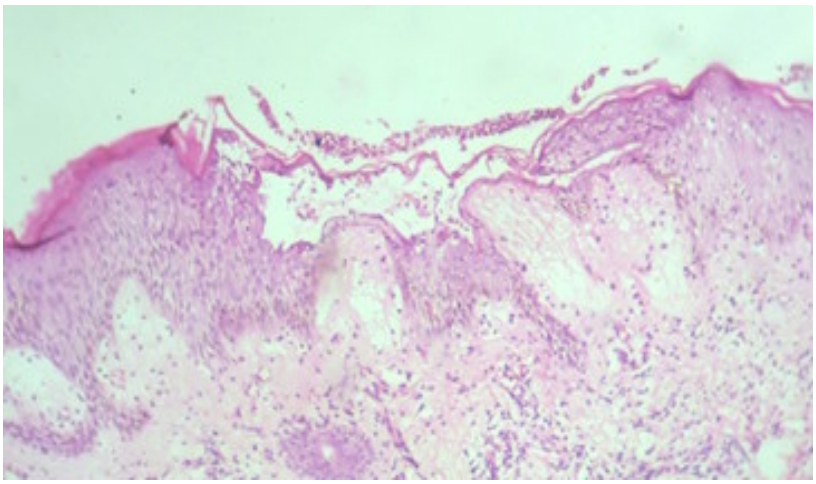


Fig.13 Blister at D:E junction with adenxal inflammation in BUllous SLE (H&E, 10X)

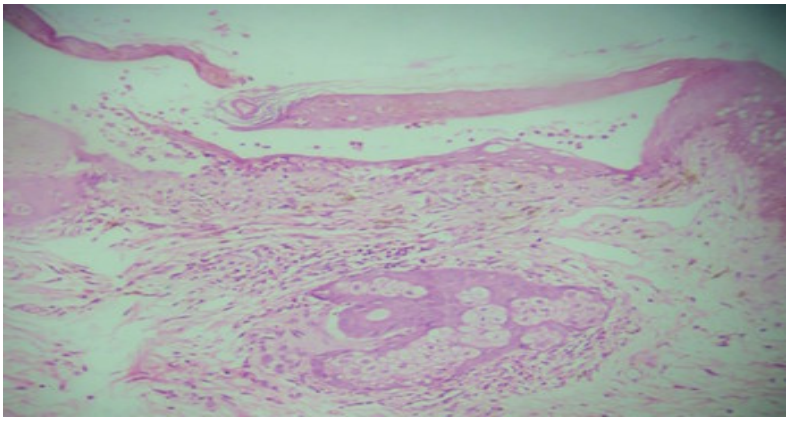


Fig14 Inflammatory cells- predominatly Neutrophilis noted in Bullous SLE (H&E, 10X)

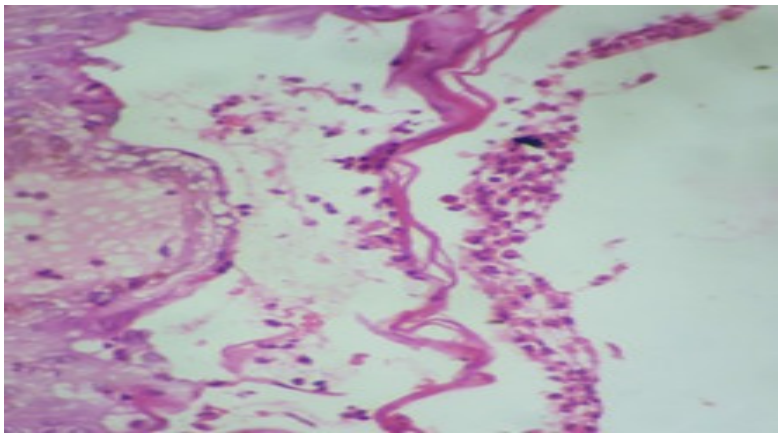
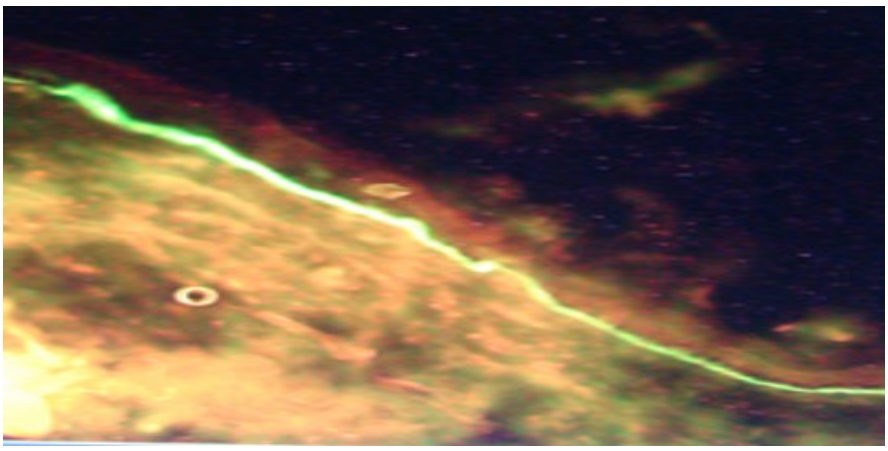


Fig.15 DIF showing Linear deposition of IgG at D:E junction in Bullous SLE (DIF, 10X)



Spongiotic dermatitis

Four cases of vesiculobullous lesion presented with spongiotic dermatitis in present study with age group of patients. M/F ratio is 1:1. Patient presented with bulla pruritc pustule similar to case study by Abreu VAN et al (2011) study in which a 15 year old female presented with intense prurtic rash.

HPE: Present study showed all cases with intraepidermal blister and spongiosis. Dyskeratotic and apoptotic keratinocyes were seen in 3 out of 4 cases. Dermal infiltration with mixed inflammatory cell infiltrate with predominant neutrophilic infiltration in all cases similar to Abreu VAN et al (2011) study which showed early evidence of a subepidermal blistering disorder, although frank blister formation was not observed. The dermis displayed a florid, superficial and deep perivascular and interstitial infiltrate of lymphocytes, histiocytes, eosinophils, neutrophils and mast cells.

Conclusion

Vesiculobullous disorders represent a heterogeneous group of dermatoses with protean manifestations. Classified according to location as suprabasal, intraepidermal, sub corneal and sub epidermal group. Pemphigus vulgaris constituted the most common subtype of vesiculobullous disorder in this study followed by bullous pemphigoid. Clinical examination of skin bullous lesion provide dermatologist gross morphological finding upon which differential diagnosis can be found out. Immunofluorescence techniques are essential to supplement clinical findings and histopathology in the diagnosis of the immunobullous disorders.

In pemphigus vulgaris and pemhigus foliaceus both show same pattern in DIF. Hence definite diagnosis cannot be made without help of histopathology. Thus DIF is just a supplement not substitute. Considering the economical constrain of DIF, clinical and histomorphological study of vesiculobullous diseases can be still used in confirming the diagnosis of diseases.

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