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Research Article

Histopathological Spectrum of Skin Disorders with Diagnostic Significance of Direct Immunofluorescence in Autoimmune Disease

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ABSTRACT

INTRODUCTION- The skin, being the largest organ of the human body, performs multiple vital functions but is prone to a wide spectrum of diseases across all age groups. Dermatological lesions range from simple inflammatory to complex neoplastic conditions. While histopathological examination remains the cornerstone for accurate diagnosis, Direct Immunofluorescence (DIF) provides an indispensable diagnostic adjunct, especially in differentiating autoimmune blistering and connective tissue diseases, where conventional histopathology alone may be insufficient. DIF enhances diagnostic precision by detecting tissue-bound immunoreactants, crucial for confirming autoimmune pathogenesis.

AIM AND OBJECTIVES- The present study was undertaken to evaluate the histopathological spectrum of dermatological lesions, analyze their age and sex distribution, identify prevalent categories of skin diseases, and highlight the diagnostic utility of DIF in autoimmune dermatoses.

MATERIALS AND METHODS- This retrospective cross-sectional study was conducted in the Department of Pathology, MGMIHS, Navi Mumbai, over a period of 10 months. A total of 63 skin biopsies were examined using routine Hematoxylin and Eosin staining, supplemented with special stains and Direct Immunofluorescence wherever indicated, particularly in suspected autoimmune lesions.

RESULT- Out of 63 cases analyzed, 55% were males and 45% females, with the highest incidence in the third decade (15 cases). The most common group was infectious dermatoses (19 cases), followed by inflammatory (17 cases) and neoplastic lesions (16 cases). Five cases of autoimmune diseases were subjected to DIF, which significantly contributed to their definitive diagnosis by identifying specific immunoreactant deposition patterns. Other diagnoses included vascular (2), pigment (1), genetic (1) and others (2).

CONCLUSION- This study reaffirms that histopathology, complemented by Direct Immunofluorescence, is indispensable in the diagnostic workup of dermatological lesions. While H&E remains fundamental, DIF serves as a critical adjunct in diagnosing autoimmune skin diseases, providing diagnostic accuracy in morphologically overlapping entities. Integrating DIF into routine dermatopathological evaluation enhances diagnostic confidence, guides appropriate therapy, and aids in understanding disease prevalence across demographic profiles.

Keywords*: Skin biopsy, Direct Immunofluorescence, Autoimmune skin diseases, Histopathological examination, Dermatopathology.*

INTRODUCTION

The skin is the largest organ of the human integumentary system, which also includes accessory structures such as hair, glands, and nails. Acting as a protective barrier against the external environment, the skin is highly susceptible to various disease-causing microorganisms and physical injuries. Skin disorders encompass a wide spectrum, ranging from inflammatory conditions to aggressive malignant neoplasms.[2]

The clinical presentation of skin conditions is often limited to features such as hyperpigmentation, hypopigmentation, macules, papules, nodules, and a few others. Many skin lesions closely resemble each other clinically, making accurate diagnosis challenging. Factors such as anatomical location, lesion type, duration, number, and associated conditions provide essential clues for confirming the diagnosis. However, histopathological examination remains the gold standard diagnostic tool for validating clinical suspicions and ensuring precise diagnosis.[1]

Despite its utility, **routine histopathology alone may not suffice in certain dermatoses, especially vesiculobullous, autoimmune, and connective tissue disorders**. In such cases, **Direct Immunofluorescence (DIF)** serves as an invaluable adjunct, helping to visualize in-situ deposition of immunoreactants like IgG, IgA, IgM, C3, and fibrinogen at specific anatomical sites within the skin. Recent studies emphasize that **DIF enhances diagnostic accuracy in autoimmune blistering disorders and other dermatoses where histology is inconclusive**[13, 14]. Newer approaches such as **serration pattern analysis** further refine the diagnostic yield of DIF.[15]

Skin lesions can be classified as following – Inflammatory, Infectious, Neoplastic (Benign and Malignant), Autoimmune, Vascular, Genetic, Pigmentary and Others[3]

AIM

To analyze and classify skin lesions based on demographic and histopathological characteristics for accurate diagnosis and understanding of disease patterns and Direct Immunofluorescence in autoimmune skin disorders.

OBJECTIVES

1. To classify skin lesions and analyze their epidemiological profile.
2. To study histopathological characteristics of skin lesions.
3. To evaluate the diagnostic role and immunofluorescence patterns of DIF in autoimmune skin disorders.

MATERIALS AND METHODS

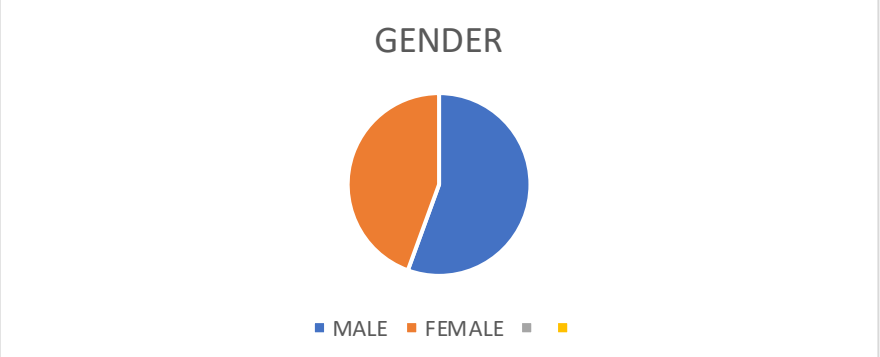
This hospital-based cross-sectional study was conducted in the Department of Pathology, MGM Medical College and Hospital, Navi Mumbai, Raigad district over a period of 10 months from March 2024 to December 2024. The study was carried out on skin punch biopsies of patients of all age group, coming to the histopathology laboratory and all cases coming during the study period. A total of 63 cases were selected as sample size on the basis of inclusion and exclusion criteria. The punch biopsy specimens were received in the 10% formalin and were allowed to fix for 24 hours. The biopsy bit was submitted whole, processed in automated tissue processor for routine paraffin embedding. Tissue sections of 5 -micron thickness were cut by microtomy. All cases were analyzed by examining Hematoxylin and Eosin -stained slides. FF stain was used in all the suspected cases of leprosy. The biopsy specimen for the suspected cases with autoimmune diseases were received in michel`s media also for Direct Immunofluorescene.

Inclusion and Exclusion criteria: All skin biopsies that showed definite signs of any specific pathology received in histopathology laboratory of the Department of Pathology during the study period were included. Inadequate/Inconclusive skin biopsies that did not show definite signs of any specific pathology were excluded from this study[4]

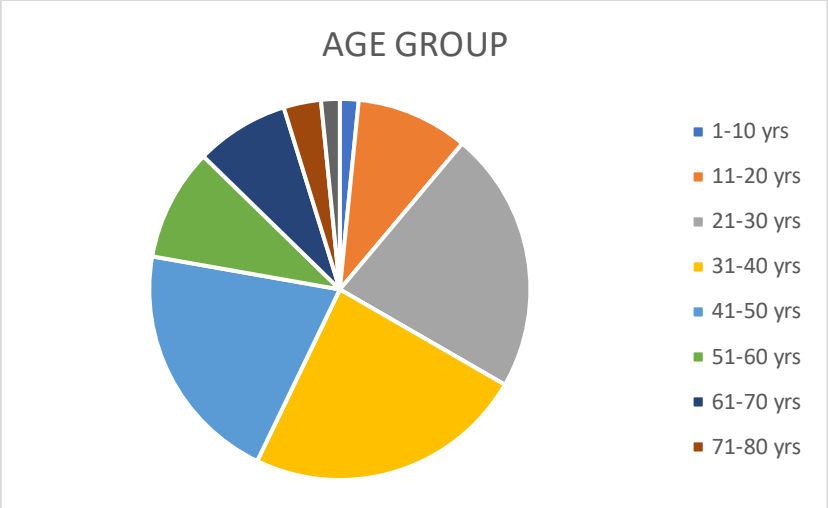
Ethicalconsideration

All the procedures performed were in accordance with the ethical standards of the institution.

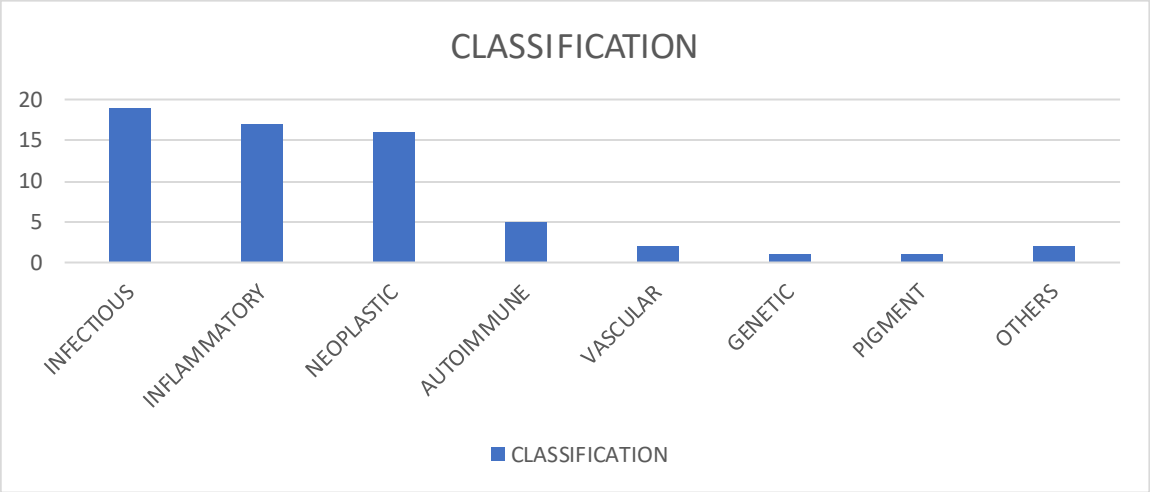
OBSERVATION AND RESULTS



GENDER	NO. OF CASES	PERCENTAGE
MALE	35	55%
FEMALE	28	45%



AGE GROUP (yrs)	NO. OF CASES (63)	PERCENTAGE
1-10	1	1.6 %
11-20	6	9.5 %
21-30	14	22.2 %
31-40	15	23.8 %
41-50	13	20.6 %
51-60	6	9.5 %
61-70	5	7.9 %
71-80	2	3.2 %
81-90	1	1.6 %

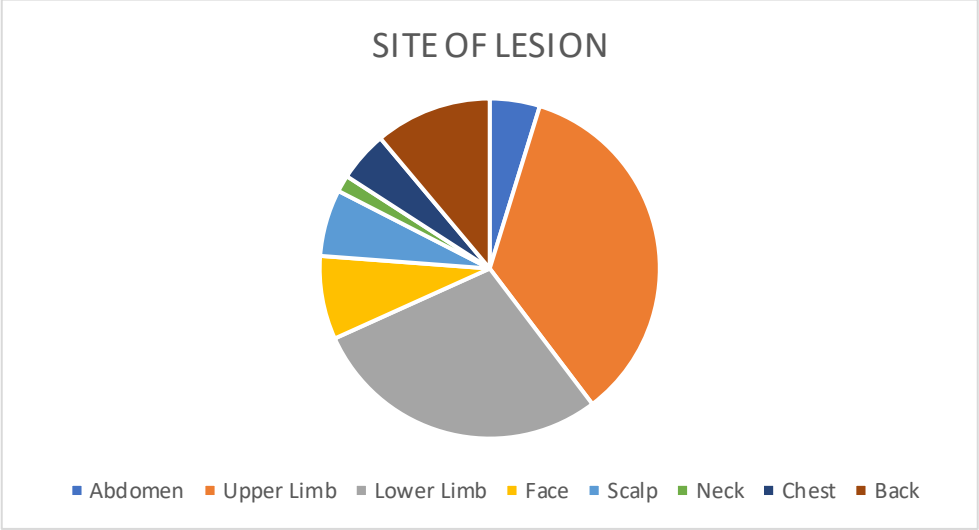


	CLASSIFICATION	PERCENTAGE
INFECTIOUS	19	30%
INFLAMMATORY	17	26.9%
NEOPLASTIC	16	25.41%
AUTOIMMUNE	5	7.94%
VASCULAR	2	3.17%
GENETIC	1	1.59%
PIGMENT	1	1.59%
OTHERS	2	3.17%
TOTAL	63	

CLASSIFICATION	NO. OF CASES	PERCENTAGE	
Infectious	**19**	**30%**	
Lepromatous leprosy	5		Fite Foracco- Contributory
Tuberculoid leprosy	6		Fite Foracco- Non-Contributory
Indeterminate leprosy	2		Fite Faraco- Non-Contributory
Erythema nodosum leprosum	2		Fite Foracco-Contributory
Borderline lepromatous leprosy	1		Fite Foracco- Non-Contributory
Granulomatous lesion, most likely TB origin (lupus vulgaris)	1		Modified ZN stain-Contributory
Verruca vulgaris	1		
Cellulitis with pseudoepitheliomatous hyperplasia	1		
Inflammatory	**17**	**26.9%**	
Lichen sclerosis	1		
Atopic dermatitis	1		
Vesicobullous lesion	1		
Vesicobullous lesion with epidermolysis secondary to drug reaction	1		
Prurigo nodularis	2		
Lichenoid polymorphous light reaction	1		
Blaschkitis with lichenoid features	1		
Pseudoepitheliomatous hyperplasia with chronic inflammation	1		
Drug rash	1		
Fixed drug eruption	1		
Lichen planus	1		
Bullous drug reaction	1		
Paniculitis with papillary endothelial hyperplasia in subcutis	1		
Erythema multiforme	1		
Urticaria	1		
Moderate perivascular inflammation with dermal fibrosis	1		
Neoplastic	**16**	**25.41%**	
Benign			
Benign soft tissue lesion (e.g., hibernoma, xanthoma)	1		
Dermal nevus	2		
Cutaneous leiomyoma	2		
Fibroepithelial polyp	1		
Fibrolipoma	1		
Benign adnexal lesion (e.g., spiradenoma, eccrine poroma)	1		
Capillary hemangioma	1		
Proliferative trichilemmal tumor	1		
Neurofibroma	1		
Angiokeratoma	1		
Benign spindle cell lesion	1		
Malignant			
Hematolymphoid malignancy	1		IHC
Dermatofibrosarcoma protuberans	1		
Bowenoid papulosis	1		
Autoimmune	**5**	**7.94%**	
Ig A pemphigus	1		DIF- Intercellular staining with IgA

Bullous dermatosis	1		Dif- confirmed it to be bullous pemphigoid, Linear at BMZ with IgG and C3
Lupus erythematosus	2		Dif (bullous le)- Granular BMZ C3 and IgG
Pemphigus vulgaris	1		Dif- fishnet pattern in IgG
Vascular	**2**	**3.17%**	
Urticarial vasculitis	1		
Superficial vascular dermatitis	1		
Genetic	**1**	**1.59%**	
Epidermolysis bullosa pruriginosa	1		DIF- negative (exclude other blistering cond) Adv- Genetic testing (COL7A1 mutation) and Electron microscopy
Pigment	**1**	**1.59%**	
Common blue nevus	1		
Others	**2**	**3.17%**	
Corn	2		
Total	**63**		

The study highlights that while infectious dermatoses, particularly leprosy, were the most common diagnosis, autoimmune diseases, although less frequent, required Direct Immunofluorescence (DIF) for definitive diagnosis. The diagnostic synergy of histopathology with adjunctive techniques like DIF and special stains (Fite-Faraco, Modified ZN) proved invaluable in the comprehensive evaluation of dermatological lesions.



SITE OF LESION	NO. OF CASES (63)	PERCENTAGE
Abdomen	3	4.8 %
Upper Limb	22	34.9 %
Lower Limb	18	28.6 %
Face	5	7.9 %
Scalp	4	6.35 %
Neck	1	1.59 %
Chest	3	4.76 %
Back	7	11.11 %

IMAGE GALLERY
H&E & SPECIAL Stain-

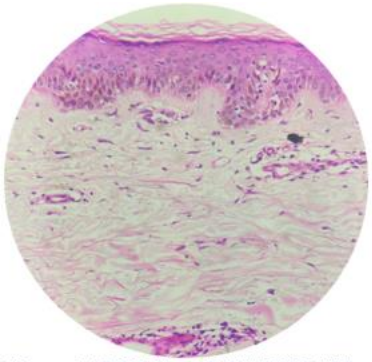


Figure 1- LEPROMATOUS LEPROSY

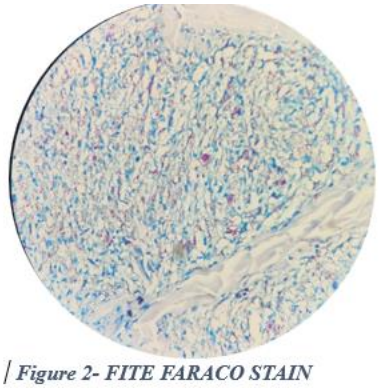


Figure 2- FITE FARACO STAIN

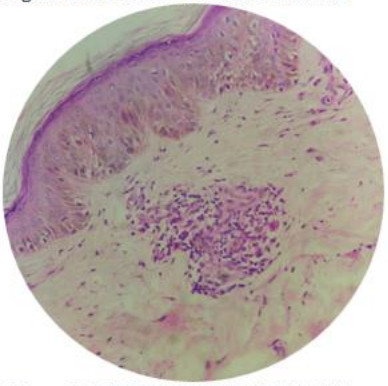


Figure 3- TUBERCULOID LEPROSY

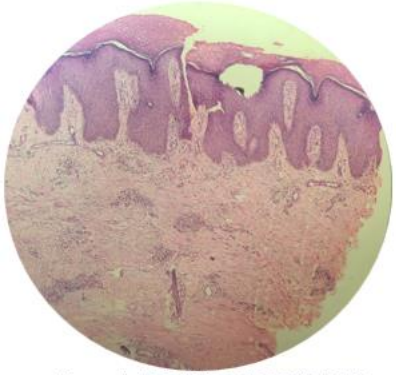


Figure 4- PRURIGO NODULARIS

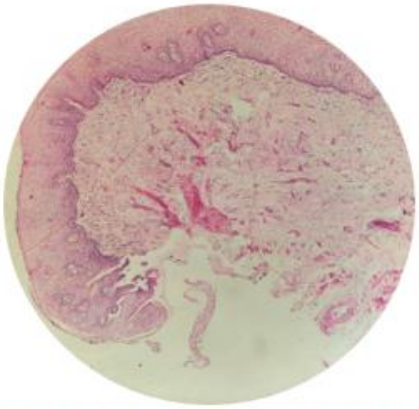


Figure 4- HEMATOLYMPHOID MALIGNANCY

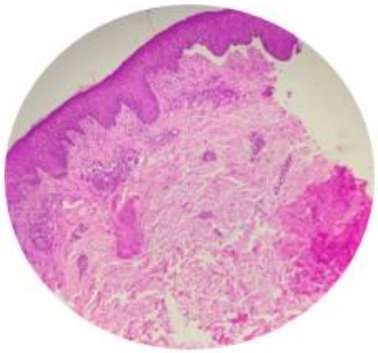


Figure 6- BULLOUS PEMPHIGOID

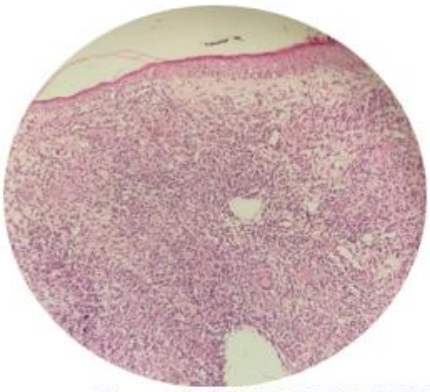


Figure 7- GRANULOMATOUS LESION

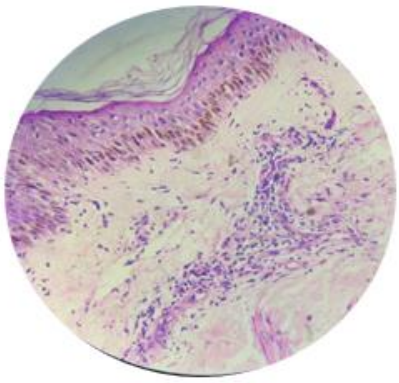
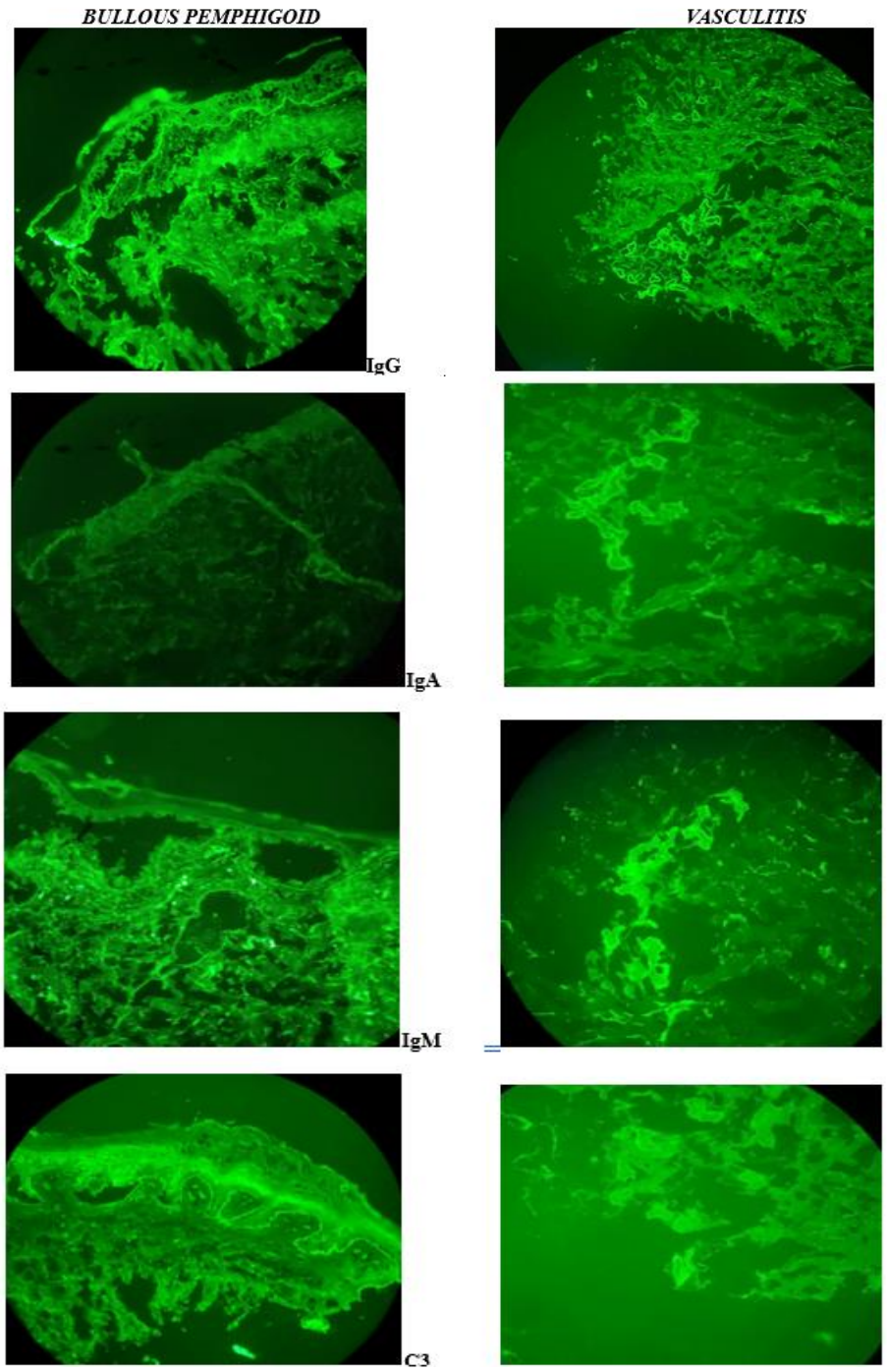


Figure 8- VASCULITIS

DIRECT IMMUNOFLUORESCENCE



DISCUSSION

In present study, out of 63 cases 35 were males and 28 were females. We found male predominance in our study, it was aligning with study of Dr. Bhumika et. al.[5], Dr. Sheela L Gaikwad et al.[2] and Pratibha et al.[6] In contrast, Mamtha K et al.[9] reported female predominance.

In my study, majority of the patients were in the age group of 31-40 (years), which was in concordance with the study of Dr. Sheela et al.[2] and Yalla et al. [7], others studies in the age group 21-30 years were Pratibha et al.[6] and Dr. Devyani et al. [8] while Dr. Bhumika et al.[5] (61-70 years) and Mamtha K et al.[9] (51-60 years).

The most common lesion in my study was infectious, also seen in study of Dr. Bhumika et al.[5] Pratibha et al.[6] Yalla et al.[7] Mamtha K et al.[9], while study of Dr. Devyani et al. [8] showed infectious and non infectious lesion, which was in contradictory to Dr. Sheela et al.[2] showing neoplastic as the common skin lesion.

In infectious lesions, Leprosy was the commonest lesion, 16 cases, out of which tuberculoid leprosy being the commonest which was in contradict to study of Dr. Bhumika et al.[5] having lepromatous leprosy as the commonest. The variation may be attributed to regional endemicity and patient referral patterns.

The most common site of lesion in mine study was upper limb, while in study conducted by Dr. Bhumika et al.[5] had lower limb as the commonest site of lesion.

In neoplastic lesions, benign were more common comprising of 13 cases, while malignant were 3 cases.

Importantly, while routine histopathology remains the cornerstone of dermatopathological diagnosis, Direct Immunofluorescence (DIF) serves as a critical adjunct, particularly in the evaluation of autoimmune skin disorders. DIF enables direct visualization of in vivo-bound immunoglobulins and complement deposits at characteristic locations (e.g., intercellular spaces in pemphigus, linear patterns along the dermoepidermal junction in pemphigoid), offering diagnostic specificity beyond conventional histopathology.

In the present study, Direct Immunofluorescence (DIF) was primarily employed in the evaluation of autoimmune blistering disorders. Of the five autoimmune cases, DIF was instrumental in establishing definitive diagnoses. The DIF patterns observed were as follows:

- Pemphigus vulgaris (1 case): Showed *intercellular* ("fishnet") deposition of IgG, confirming the diagnosis.
- Bullous pemphigoid (1 case): Exhibited a *linear* pattern of IgG and C3 along the basement membrane zone (BMZ).
- IgA pemphigus (1 case): Displayed *intercellular* deposition of IgA, consistent with IgA pemphigus.
- Bullous lupus erythematosus (2 cases): Revealed *granular* deposition of IgG and C3 along the BMZ.

These findings are congruent with the established immunopathological features described by Zillikens D et al.[11] in his comprehensive overview of autoimmune blistering diseases. According to Zillikens,[11] DIF remains the *gold standard* for diagnosing autoimmune blistering disorders, reliably detecting:

- Intercellular IgG/C3 in pemphigus vulgaris,
- Linear IgG/C3 at the BMZ in bullous pemphigoid,
- Granular IgA deposition at dermal papillae in dermatitis herpetiformis (though not encountered in our study),
- And granular IgG/C3 along the BMZ in lupus erythematosus.

Thus, DIF findings in our study mirror the diagnostic patterns reported by Zillikens[11], reaffirming its superior sensitivity and specificity in distinguishing autoimmune blistering diseases from other clinically overlapping dermatoses.

While Zillikens provides a broader spectrum including dermatitis herpetiformis and epidermolysis bullosa acquisita (EBA), our study reflects the utility of DIF specifically in the Indian population context, where autoimmune blistering disorders like pemphigus vulgaris and bullous pemphigoid predominate.

Study by Vaishnav et al.[12] reinforce the diagnostic relevance of DIF, mirroring the immunofluorescence patterns seen in our study—fishnet IgG deposition in pemphigus vulgaris and linear BMZ staining in bullous pemphigoid. These patterns remain consistent across populations and underscore DIF's irreplaceable role in routine dermatopathological diagnostics. The concordance between our results and global data underscores the critical, irreplaceable role of DIF in dermatopathology.

	Present study	Dr. Bhumika et al.[5]	Pratibha et al.[6]	Dr. Sheela et al.[2]	Yalla et al.[7]	Dr. Devyani et al.[8]	Mamtha K et al.[9]	Ram Chandra Adhikari et al[10]
Total Cases	63	105	50	113	150	400	286	1040
Duration of study	10 months	3 years	1 year	6 months	2 yrs	2 yrs 4 months	2 years	2 years
M : F	M>F	M>F	M>F	M>F	M>F	M>F	M<F	M>F
Age group (yrs)	31-40	61-70	21-30	31-40	31-40	21-30	51-60	31-40
Most common skin lesion	Infectious	Infectious	Infectious	Neoplastic	Infectious	Non infectious and Infectious	Infectious	Non – infectious vesicobullous

CONCLUSION

This study, conducted over 10 months and including 63 cases, reinforces the integral role of histopathological examination in diagnosing a broad spectrum of dermatological lesions. This study highlights that while infectious dermatoses, especially leprosy, constituted the predominant group of skin lesions in our setting, the role of Direct Immunofluorescence (DIF) was indispensable in resolving diagnostic uncertainty in autoimmune skin disorders.

Routine histopathology, though foundational, was insufficient to definitively differentiate autoimmune blistering diseases such as pemphigus vulgaris, bullous pemphigoid, IgA pemphigus, and bullous lupus erythematosus from other vesicobullous or inflammatory dermatoses. DIF enabled diagnostic clarity by revealing disease-specific immunoreactant patterns — such as intercellular IgG in pemphigus, linear IgG/C3 at the BMZ in bullous pemphigoid, and granular IgG/C3 deposits in lupus erythematosus.

These findings reinforce that DIF not only complements but transforms dermatopathological diagnosis by providing specificity in autoimmune conditions with overlapping histological appearances. Furthermore, DIF helped to:

- Avoid misclassification of blistering dermatoses,
- Guide appropriate therapeutic interventions, and
- Prompt further investigations such as genetic testing in suspected inherited disorders (e.g., epidermolysis bullosa).

Given the high specificity and diagnostic yield of DIF in autoimmune dermatoses, its incorporation as a routine adjunct in dermatopathological workups is strongly recommended, especially in regions with a high burden of autoimmune blistering diseases.

By integrating histopathology with DIF and adjunctive special stains, dermatopathological diagnosis can achieve greater diagnostic precision, leading to improved patient care and outcomes.

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