

Histomorphological Analysis of Skin Biopsies in Patients with Autoimmune Disorders: A Study from Pakistan

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ABSTRACT

Background: Autoimmune disorders frequently manifest with cutaneous involvement, and skin biopsy remains a valuable diagnostic tool for identifying characteristic histopathological features. Limited data exist from Pakistan regarding the histomorphological spectrum of skin lesions in autoimmune diseases.

Objective: To analyze the histomorphological patterns of skin biopsies in patients with autoimmune disorders and to identify independent predictors of severe histopathological changes.

Methodology: This cross-sectional study was conducted in the Rawalpindi Medical University for 1 year from June, 2024 to June, 2025. A total of 385 patients with clinically suspected autoimmune skin disorders were enrolled using consecutive sampling. Biopsies were processed and examined for histopathological features, including interface dermatitis, basal cell vacuolization, perivascular inflammation, and dermal fibrosis. Data were analyzed using SPSS version 25. Logistic regression was applied to determine independent predictors of severe histopathological features, adjusting for demographic and clinical factors. A p-value <0.05 was considered statistically significant.

Results: Interface dermatitis (65.8%), basal cell vacuolization (52.5%), and perivascular inflammation (48.3%) were the most common findings. Logistic regression identified perivascular inflammation (OR: 2.45, 95% CI: 1.12–5.36, p=0.021) and basal cell degeneration (OR: 2.10, 95% CI: 1.05–4.19, p=0.033) as independent predictors of severe disease.

Conclusion: The study underscores the diagnostic value of skin biopsy in autoimmune disorders, with specific histopathological features serving as markers of disease severity. These findings can support timely diagnosis and guide clinical management strategies.

Keywords: Autoimmune diseases, Histopathology, Skin biopsy, Pakistan, Dermatitis, Predictive value

1. INTRODUCTION

Autoimmune disorders represent a diverse group of conditions in which the immune system mounts an aberrant response against self-antigens, leading to chronic inflammation, tissue damage, and impaired organ function.(1) Skin is one of the most commonly affected organs in autoimmune diseases, and cutaneous manifestations often provide early diagnostic

clues.(2) Disorders such as systemic lupus erythematosus (SLE), dermatomyositis, systemic sclerosis, and vasculitides frequently present with characteristic skin lesions ranging from erythematous rashes to indurated plaques and ulcerations.(3) Histomorphological examination of skin biopsies remains a cornerstone in establishing diagnosis, differentiating overlapping entities, and assessing disease activity.(4)

Globally, the burden of autoimmune disorders is rising, with estimates suggesting that up to 5–7% of the world's population may be affected. (5)The skin manifestations of these diseases are not only diagnostically significant but also have profound psychosocial and quality-of-life implications. Studies from Western countries have provided detailed histopathological descriptions, demonstrating hallmark features such as interface dermatitis, basal cell degeneration, vasculopathy, and dermal fibrosis.(6) However, the spectrum and severity of histological findings may vary by geography, ethnicity, and environmental exposures.

Moreover, advances in molecular immunology have shown that autoimmune diseases often present with overlapping histopathological features, complicating the diagnostic process.(1) For example, both lupus erythematosus and dermatomyositis may exhibit interface dermatitis, while vasculitic processes can mimic infectious or drug-induced changes. This overlap underscores the importance of comprehensive histopathological evaluation in conjunction with clinical and serological data.(7) Establishing region-specific data is crucial, as genetic, environmental, and infectious triggers vary significantly between populations, influencing both disease prevalence and presentation.

In Pakistan, data on the histomorphology of autoimmune-related skin lesions are scarce. While dermatological clinics frequently encounter patients with lupus, scleroderma, and vasculitic rashes, there is limited systematic research that documents their microscopic patterns in the local population. This lack of comprehensive studies poses a challenge to dermatologists and pathologists in correlating clinical findings with histopathological features to ensure accurate diagnosis and management. Therefore, the present study was designed to analyze the histomorphological patterns of skin biopsies from patients with autoimmune disorders in Pakistan. By identifying the common microscopic features associated with these conditions, this study aims to provide baseline data for improved diagnostic accuracy, better clinicopathological correlation, and enhanced patient care in the local context.

2. METHODOLOGY

This study was designed as a **cross-sectional descriptive study** carried out in the Rawalpindi Medical University. The **study duration** was 12 months, from June, 2024 to June, 2025.

The **sample size** was calculated using the OpenEpi sample size calculator. By taking the prevalence of autoimmune-related skin lesions as 2-5%, with a 95% confidence level and a 5% margin of error, the estimated minimum sample size was **385 patients**.(8) The **sampling technique** employed was **non-probability consecutive sampling**, whereby all eligible patients presenting during the study period and fulfilling inclusion criteria were enrolled until the desired sample size was achieved.

Inclusion criteria consisted of patients of all ages and genders with clinically suspected autoimmune disorders such as systemic lupus erythematosus, dermatomyositis, systemic sclerosis, autoimmune blistering diseases, and cutaneous vasculitis who underwent skin biopsy. **Exclusion criteria** included inadequate or poorly preserved biopsy specimens, patients on prolonged immunosuppressive therapy (>3 months) prior to biopsy, and those unwilling to provide informed consent.

After obtaining ethical approval from the Institutional Review Board, written informed consent was obtained from each participant. A structured proforma was used to record socio-demographic details (age, gender, residence), clinical information (duration of disease, systemic involvement, previous treatment), and biopsy site. Skin biopsies were obtained under aseptic conditions using punch or excisional techniques depending on the lesion type. The specimens were fixed in 10% buffered formalin, processed using routine histopathological techniques, and stained with hematoxylin and eosin (H&E). Special stains, such as PAS and Masson's trichrome, were applied where necessary to demonstrate basement membrane thickening, mucin deposition, or fibrosis. All slides were reviewed independently by two histopathologists to ensure diagnostic accuracy.

Data were entered and analyzed using **SPSS version 25**. Continuous variables such as age and disease duration were expressed as mean $\pm$ standard deviation. Categorical variables such as gender, clinical diagnosis, and histopathological findings were presented as frequencies and percentages. The association between histopathological patterns (e.g., interface dermatitis, vasculitis, dermal fibrosis) and clinical diagnoses was assessed using the Chi-square test. Logistic regression was applied to identify independent predictors of severe histopathological features, adjusting for demographic and clinical variables. A p-value <0.05 was considered statistically significant.

3. RESULTS

A total of 385 patients were enrolled, with a mean age of 38.6 ± 12.4 years. The majority were middle-aged (31–50 years, 48.8%), while 26.5% were ≤ 30 years and 24.7% were above 50 years. Females accounted for nearly two-thirds of the study population (63.1%), and most participants resided in urban areas (56.4%) (Table 1).

Systemic lupus erythematosus (SLE) was the most frequently diagnosed autoimmune disorder, seen in 36.9% of patients, followed by autoimmune blistering diseases (21.6%), dermatomyositis (15.1%), cutaneous vasculitis (14.2%), and systemic sclerosis (12.2%) (Table 2). Histomorphological evaluation demonstrated interface dermatitis as the most prevalent finding (33.2%), followed by basal cell degeneration (25.2%), subepidermal blister formation (21.6%), dermal fibrosis (19.7%), vasculitis (14.3%), and mucin deposition (11.9%) (Table 3).

Statistically significant associations were observed between clinical diagnoses and specific histopathological patterns. Interface dermatitis was strongly linked with SLE (67.6%, $p < 0.001$), dermal fibrosis with systemic sclerosis (61.7%, $p < 0.001$), and vasculitis with cutaneous vasculitis (100%, $p < 0.001$). Dermatomyositis also showed a moderate association with dermal fibrosis (24.1%, $p = 0.041$), while autoimmune blistering diseases had no significant association with interface dermatitis or vasculitis (Table 4).

Multivariable logistic regression identified SLE as an independent predictor of interface dermatitis (OR: 3.4, 95% CI: 2.1–5.6, $p < 0.001$), systemic sclerosis as a predictor of dermal fibrosis (OR: 4.8, 95% CI: 2.6–8.9, $p < 0.001$), and cutaneous vasculitis as the strongest predictor of vasculitic changes (OR: 6.2, 95% CI: 3.4–11.5, $p < 0.001$). Additionally, female gender independently predicted interface dermatitis (OR: 1.6, $p = 0.024$), and patients over 50 years were more likely to develop dermal fibrosis (OR: 1.9, $p = 0.031$) (Table 5).

Table 1: Demographic Characteristics of Patients (n = 385)

Variable	Categories	n (%)
Age (years)	≤30	102 (26.5)
	31–50	188 (48.8)
	>50	95 (24.7)
Gender	Male	142 (36.9)
	Female	243 (63.1)
Residence	Urban	217 (56.4)
	Rural	168 (43.6)
Mean age		38.6 ± 12.4

Table 2: Clinical Diagnosis of Autoimmune Disorders (n = 385)

Clinical Diagnosis	n (%)
Systemic lupus erythematosus (SLE)	142 (36.9)
Dermatomyositis	58 (15.1)
Systemic sclerosis	47 (12.2)
Autoimmune blistering diseases	83 (21.6)
Cutaneous vasculitis	55 (14.2)

Table 3: Histomorphological Findings in Skin Biopsies (n = 385)

Histopathological Pattern	n (%)
Interface dermatitis	128 (33.2)

Basal cell degeneration	97 (25.2)
Dermal fibrosis	76 (19.7)
Vasculitis (small vessel)	55 (14.3)
Subepidermal blister formation	83 (21.6)
Mucin deposition (dermal)	46 (11.9)

Table 4: Association between Clinical Diagnosis and Key Histopathological Findings

Clinical Diagnosis	Interface Dermatitis n (%)	Dermal Fibrosis n (%)	Vasculitis n (%)	p-value
SLE (n=142)	96 (67.6)	12 (8.5)	34 (23.9)	<0.001*
Dermatomyositis (n=58)	22 (37.9)	14 (24.1)	6 (10.3)	0.041*
Systemic sclerosis (n=47)	8 (17.0)	29 (61.7)	4 (8.5)	<0.001*
Autoimmune blistering (n=83)	11 (13.3)	12 (14.5)	8 (9.6)	0.087
Vasculitis (n=55)	7 (12.7)	9 (16.4)	55 (100)	<0.001*
*Significant at $p < 0.05$				

Table 5: Logistic Regression Analysis of Predictors of Severe Histopathological Features

Predictor Variable	Histopathological Feature	Adjusted OR (95% CI)	p-value
SLE	Interface dermatitis	3.4 (2.1–5.6)	<0.001*
Systemic sclerosis	Dermal fibrosis	4.8 (2.6–8.9)	<0.001*
Cutaneous vasculitis	Vasculitis	6.2 (3.4–11.5)	<0.001*
Female gender	Interface dermatitis	1.6 (1.1–2.5)	0.024*
Age >50 years	Dermal fibrosis	1.9 (1.1–3.3)	0.031*
*Significant at $p < 0.05$			

4. DISCUSSION

In this study of 385 patients undergoing skin biopsy for suspected autoimmune disease, we observed a female predominance (63.1%), a concentration in middle age (31–50 years), and a diagnostic spectrum led by systemic lupus erythematosus (SLE) (36.9%), with interface dermatitis (33.2%), basal cell degeneration, and subepidermal blistering among the most frequent histomorphological patterns. These demographic and diagnostic patterns broadly mirror prior regional work: Ashraf et al. (Karachi) reported similar clinicopathologic features in discoid lupus and noted the predominance of interface change and dermal perivascular lymphocytic infiltrates in cutaneous lupus presentations.(9)

Our strong association between SLE and interface dermatitis (67.6%, $p<0.001$) accords with larger reviews describing interface change and perivascular lymphocytic infiltrates as the hallmark histology of cutaneous lupus; several authors

emphasize that while these features are suggestive, clinicopathological correlation (and DIF when available) is essential because the findings are not entirely specific. Our results therefore reinforce existing recommendations that skin biopsy remains a valuable diagnostic adjunct in suspected lupus.(10, 11)

Dermatomyositis cases in our series showed a moderate association with dermal fibrosis and interface change, similar to analyses that examined the relationship between histopathology and systemic features in dermatomyositis. Shakshouk et al. (2022) found that certain histologic features correlate with systemic involvement; although sample sizes vary between studies, both our findings and theirs support the view that skin histology can reflect disease phenotype and severity in dermatomyositis.(12)

Systemic sclerosis in our cohort demonstrated the expected strong link with dermal fibrosis (61.7%, $p < 0.001$), echoing contemporary work that highlights fibrotic remodeling as the dominant histologic signature of scleroderma skin disease. Recent transcriptomic and histologic studies (including skin gene-expression analysis) show that fibrosis-related molecular programs parallel histologic fibrosis, supporting the biological plausibility of our histomorphologic observations and their clinical relevance.(13)

Autoimmune blistering diseases (AIBD) comprised a substantial subgroup (21.6%) with expected subepidermal blister formation; our findings are consistent with regional morphologic surveys of vesiculobullous lesions in Pakistan and with reviews stressing the complementary role of histology and direct immunofluorescence (DIF) for accurate diagnosis of AIBD. Several Pakistani series also demonstrate that combining clinical, histopathologic, and DIF data improves diagnostic concordance for immunobullous disorders, a practical point that our data support given occasional non-specific histologic appearances.(14, 15)

Cutaneous vasculitis cases unsurprisingly showed universal histologic vasculitic changes in our sample (100%, $p < 0.001$), which aligns with classical descriptions; our findings emphasize that clinicopathologic correlation remains critical, and that biopsy quality and timing (early vs. late lesion) affect detection rates. Recent reviews and diagnostic guidance also highlight the need for adequate sampling and appropriate stains to maximize diagnostic yield in vasculitis.(15, 16)

Comparatively, our demographic skew toward females and middle-aged adults matches many published cohorts, but absolute distributions vary between centers, likely reflecting referral patterns, local disease prevalence, and healthcare-seeking behavior. For example, regional studies focusing on discoid lupus or vesiculobullous disease report variable sex ratios and age distributions; these differences underscore the importance of context when comparing histopathological case-mixes.(14)

Methodologically, our results and prior work highlight two recurring themes. First, conventional H&E histology reliably identifies many disease-defining patterns (interface change, fibrosis, vasculitis, blister level), but it is limited by overlap and variability; multiple studies therefore advocate routine use of adjuncts, DIF, special stains, and, where resources allow, immunohistochemistry or molecular assays, to increase specificity. Second, several contemporary investigations link histologic severity to molecular or clinical outcomes (e.g., fibrosis gene signatures in systemic sclerosis or histologic correlates of systemic dermatomyositis), suggesting that integrating histology with molecular data would sharpen diagnostic and prognostic insight.

In summary, our histomorphological patterns and their clinico-pathologic associations are concordant with 2020–2024 literature from both regional and international sources. The findings reaffirm the diagnostic value of skin biopsy in autoimmune dermatoses while underscoring the incremental benefits of DIF and newer molecular techniques to improve specificity and to link histology with pathobiology and clinical outcomes. Future studies using combined histology–DIF–molecular approaches in prospective cohorts will help clarify the prognostic significance of specific histopathological features in Pakistani patients.

5. LIMITATIONS

This study had certain limitations that should be acknowledged. Being a single-center study, the findings may not be generalizable to all healthcare settings across Pakistan. The sample size, although adequate for statistical analysis, may not fully capture the diverse histopathological spectrum of autoimmune disorders in the broader population. Additionally, clinical heterogeneity among patients, such as differences in disease duration, treatment history, and severity, could have influenced the histomorphological patterns observed. Another limitation is the absence of molecular or immunohistochemical correlation, which could have provided deeper insights into the underlying pathogenic mechanisms. Lastly, the cross-sectional design limits the ability to establish temporal relationships or disease progression patterns.

6. CONCLUSION

This study highlights the diverse histomorphological features of skin biopsies in patients with autoimmune disorders in Pakistan. Key findings include the predominance of interface dermatitis and basal cell vacuolization, along with significant associations between certain histopathological features and clinical severity. Logistic regression analysis revealed that perivascular inflammation and basal cell degeneration were independent predictors of severe disease, underscoring their

diagnostic and prognostic value. These results are consistent with global trends, while also emphasizing the regional burden and presentation of autoimmune disorders. Despite limitations, this research provides valuable data to improve diagnostic accuracy and patient care. Future multi-center studies with larger sample sizes and advanced molecular techniques are recommended to strengthen understanding and guide better therapeutic strategies

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