

CORRELATION OF HISTOPATHOLOGICAL FINDINGS WITH BACTERIAL CULTURE AND INFLAMMATORY MARKERS IN DIABETIC FOOT ULCERS

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ABSTRACT

Background: Diabetic foot ulcers are among the most serious complications of diabetes mellitus and are associated with chronic infection, delayed wound healing, lower-limb amputation, and increased healthcare burden.

Objectives: To determine the correlation of histopathological findings with bacterial culture and inflammatory markers in diabetic foot ulcers.

Study Design & Setting: Analytical cross-sectional study conducted in the Department of General Surgery in collaboration with the Departments of Pathology and Microbiology at Department of Pathology, PUMHSW, Nawabshah from September 2026 to February 2026.

Methodology: A total of 120 patients with diabetic foot ulcers were enrolled using non-probability consecutive sampling. Deep tissue specimens obtained after surgical debridement were subjected to histopathological examination and aerobic bacterial culture. Blood samples were analyzed for C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and total leukocyte count (TLC). Data were analyzed using SPSS version 27. Correlation between histopathological findings, bacterial culture, and inflammatory markers was determined using Pearson's or Spearman's correlation coefficient, while categorical variables were compared using the Chi-square test. A p-value of ≤ 0.05 was considered statistically significant.

Results: The mean age of the patients was 56.8 ± 10.9 years, and 63.3% were male. Chronic inflammation (43.3%) was the most common histopathological finding, while tissue necrosis was observed in 65.8% of cases. Positive bacterial cultures were identified in 84.2% of patients, with *Staphylococcus aureus* being the most frequently isolated organism (27.7%). Histopathological findings demonstrated a significant association with bacterial culture results ($p = 0.041$). Histopathological severity showed significant positive correlations with CRP ($r = 0.612$, $p < 0.001$), ESR ($r = 0.548$, $p < 0.001$), and TLC ($r = 0.471$, $p < 0.001$).

Conclusion: Histopathological findings in diabetic foot ulcers demonstrated significant correlations with bacterial culture positivity and inflammatory markers. Integrating histopathological assessment with microbiological and inflammatory marker evaluation may improve diagnostic accuracy, disease severity assessment, and clinical decision-making in the management of diabetic foot ulcers.

KEYWORDS: Bacterial culture; C-reactive protein; Diabetic foot ulcer; Histopathology; Inflammation; Osteomyelitis

INTRODUCTION

Diabetic foot ulcer (DFU) is a chronic, non-healing lesion involving the skin and underlying tissues of the foot in patients with diabetes mellitus, usually occurring as a consequence of peripheral neuropathy, peripheral arterial disease, and repetitive trauma. It represents one of the most serious and costly complications of diabetes because it frequently progresses to infection, gangrene, lower-limb amputation, prolonged hospitalization, and increased mortality.^{1,2} The chronic hyperglycemic state impairs leukocyte function, collagen synthesis, angiogenesis, and wound healing, creating an environment that favors persistent inflammation and microbial colonization. Consequently, diabetic foot ulcers impose a substantial burden on

healthcare systems and significantly reduce patients' quality of life.^{3,4} According to a global meta-analysis, the prevalence of diabetic foot ulcers among individuals with diabetes is approximately 6.3%, and nearly one in six patients with diabetes is expected to develop a foot ulcer during their lifetime. Furthermore, almost half of diabetic foot ulcers become clinically infected, markedly increasing the risk of limb loss and death.⁵

The burden of diabetic foot disease is particularly high in low- and middle-income countries where delayed presentation, limited access to multidisciplinary care, and inappropriate antibiotic use contribute to poor outcomes. Pakistan is among the countries with the highest prevalence of diabetes worldwide, and diabetic foot complications have become a growing public health concern.⁶ A recent systematic review and meta-analysis of 12 Pakistani studies involving 14,201 diabetic patients reported a pooled prevalence of diabetic foot ulcers of 12.16% (95% CI: 5.91–20.23%), with prevalence increasing to 19.54% in studies published between 2011 and 2022. The review also highlighted marked regional variation, emphasizing the need for improved preventive strategies and evidence-based management protocols for diabetic foot disease in Pakistan.⁷

Infected diabetic foot ulcers are characterized by complex interactions between tissue destruction, bacterial invasion, and host inflammatory responses. Histopathological examination provides objective evidence regarding the severity of inflammation, tissue necrosis, granulation tissue formation, vascular compromise, and the presence of osteomyelitis, making it an important adjunct in the evaluation of diabetic foot infections.^{8,9} Simultaneously, bacterial culture remains the gold standard for identifying causative organisms and guiding targeted antimicrobial therapy, although culture findings alone may not accurately reflect the extent of tissue damage or inflammatory activity.^{10,11} Inflammatory biomarkers such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and total leukocyte count (TLC) are widely used to assess systemic inflammation and monitor disease severity; however, their relationship with local histopathological changes remains incompletely understood.¹²

Correlating histopathological findings with bacterial culture results and inflammatory markers may improve diagnostic accuracy, facilitate early recognition of severe infection, optimize antimicrobial selection, and assist clinicians in determining the need for surgical debridement or amputation. Establishing these correlations may ultimately contribute to better risk stratification, improved limb salvage, reduced healthcare costs, and enhanced clinical outcomes in patients with diabetic foot ulcers

METHODS

This analytical cross-sectional study was conducted in the Departments of Pathology and Microbiology at Department of Pathology, PUMHSW, Nawabshah from September 2026 to February 2026 after approval from the Institutional Ethical Review Committee. A total of 120 patients with diabetic foot ulcers fulfilling the eligibility criteria were enrolled using a non-probability consecutive sampling technique. The sample size of 120 patients was calculated using the WHO sample size calculator by considering a 95% confidence level, 8% margin of error, and an anticipated correlation based on previous published literature.¹³

Adult patients aged 18 years or above with type 1 or type 2 diabetes mellitus presenting with clinically diagnosed diabetic foot ulcers requiring debridement or biopsy were included in the study. Patients who had received systemic antibiotics for more than 72 hours before presentation, had traumatic foot ulcers, venous ulcers, pressure ulcers, malignancy involving the ulcer, autoimmune diseases, immunosuppressive therapy, or refused consent were excluded from the study.

After obtaining written informed consent, demographic and clinical data including age, gender, duration of diabetes, duration of ulcer, glycemic status, comorbidities, Wagner grade, and ulcer characteristics were recorded on a structured proforma. Venous blood samples were collected before initiation of definitive treatment for estimation of inflammatory markers including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), total leukocyte count (TLC), and neutrophil percentage using the hospital laboratory according to standardized protocols. Deep tissue specimens were obtained from the base of the ulcer after adequate wound cleansing and surgical debridement under aseptic conditions. One portion of the specimen was sent for aerobic bacterial culture and antimicrobial susceptibility testing, while the second portion was fixed in 10% buffered formalin and sent for histopathological examination. Histopathological assessment was performed by consultant histopathologists who evaluated inflammatory cell infiltrate, tissue necrosis, granulation tissue formation, fibrosis, vascular changes, and evidence of osteomyelitis using standard histopathological criteria.

All laboratory investigations were performed in the same hospital laboratories to minimize inter-laboratory variation. Data were entered and analyzed using Statistical Package for Social Sciences (SPSS) version 27. Quantitative variables were expressed as mean $\pm$ standard deviation, whereas qualitative variables were presented as frequencies and percentages. The normality of continuous variables was assessed using the Shapiro–Wilk test. The correlation between histopathological findings, bacterial culture results, and inflammatory markers was determined using Spearman's rank correlation coefficient or Pearson's correlation coefficient as appropriate. Associations between categorical variables were assessed using the Chi-square test or Fisher's exact test where applicable. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

The study included 120 patients with diabetic foot ulcers. The mean age of the participants was 56.8 ± 10.9 years. Most patients (52.5%) belonged to the 41–60 years age group, followed by those older than 60 years (35.8%), while 11.7% were aged 18–40 years. Male patients (63.3%) outnumbered female patients (36.7%). The mean duration of diabetes was 11.4 ± 5.8 years, the mean duration of the ulcer was 5.9 ± 2.8 weeks, and the mean HbA1c level was $8.9 \pm 1.4\%$, as given in Table 1.

Table 1: Baseline Demographic and Clinical Characteristics of the Study Population (n = 120)

Variable	Frequency (%) / Mean $\pm$ SD
Age (years)	56.8 ± 10.9
18–40 years	14 (11.7)
41–60 years	63 (52.5)
>60 years	43 (35.8)
Male	76 (63.3)
Female	44 (36.7)
Duration of diabetes (years)	11.4 ± 5.8
Duration of ulcer (weeks)	5.9 ± 2.8
HbA1c (%)	8.9 ± 1.4

Histopathological examination demonstrated that chronic inflammation was the most frequent finding, observed in 43.3% of patients, followed by acute-on-chronic inflammation in 33.3% and acute inflammation in 23.3%. Tissue necrosis was identified in 65.8% of cases, granulation tissue in 69.2%, and osteomyelitis in 24.2% of patients, as given in Table 2.

Table 2: Histopathological Findings in Diabetic Foot Ulcers (n = 120)

Histopathological Finding	Frequency (%)
Acute inflammation	28 (23.3)
Chronic inflammation	52 (43.3)
Acute on chronic inflammation	40 (33.3)
Tissue necrosis	79 (65.8)
Granulation tissue	83 (69.2)
Osteomyelitis	29 (24.2)

Bacterial culture was positive in 101 (84.2%) patients, whereas 19 (15.8%) patients had negative culture results. Among the positive cultures, *Staphylococcus aureus* was the most commonly isolated organism (27.7%), followed by *Pseudomonas aeruginosa* (21.8%), *Escherichia coli* (18.8%), *Klebsiella pneumoniae* (14.9%), while other bacterial isolates accounted for 16.8% of cases, as given in Table 3.

Table 3: Bacterial Culture Results in Diabetic Foot Ulcers (n = 120)

Variable	Frequency (%)
Positive culture	101 (84.2)
Negative culture	19 (15.8)
<i>Staphylococcus aureus</i>	28 (27.7)
<i>Pseudomonas aeruginosa</i>	22 (21.8)
<i>Escherichia coli</i>	19 (18.8)
<i>Klebsiella pneumoniae</i>	15 (14.9)
Other organisms	17 (16.8)

The inflammatory markers showed elevated mean values among the study participants. The mean C-reactive protein level was 71.5 ± 34.2 mg/L, the mean erythrocyte sedimentation rate was 58.9 ± 22.6 mm/hour, and the mean total leukocyte count was $12.7 \pm 3.5 \times 10^9/L$, as given in Table 4.

Table 4: Inflammatory Markers Among Study Participants (n = 120)

Variable	Mean $\pm$ SD
C-reactive protein (mg/L)	71.5 ± 34.2

Erythrocyte sedimentation rate (mm/hr)	58.9 ± 22.6
Total leukocyte count (×10 ⁹ /L)	12.7 ± 3.5

A statistically significant association was observed between histopathological findings and bacterial culture results ($p = 0.041$). Positive bacterial cultures were detected in 92.9% of patients with acute inflammation, 87.5% of those with acute-on-chronic inflammation, and 76.9% of patients with chronic inflammation, indicating that culture positivity varied according to the histopathological pattern, as given in Table 5.

Table 5: Association of Histopathological Findings with Bacterial Culture Results (n = 120)

Histopathological Finding	Culture Positive n (%)	Culture Negative n (%)	p-value
Acute inflammation (n=28)	26 (92.9)	2 (7.1)	0.041
Chronic inflammation (n=52)	40 (76.9)	12 (23.1)	
Acute on chronic inflammation (n=40)	35 (87.5)	5 (12.5)	

Histopathological severity showed a significant positive correlation with inflammatory markers. A strong positive correlation was observed with C-reactive protein ($r = 0.612$, $p < 0.001$), followed by erythrocyte sedimentation rate ($r = 0.548$, $p < 0.001$) and total leukocyte count ($r = 0.471$, $p < 0.001$), suggesting that increasing histopathological severity was associated with higher inflammatory marker levels, as given in Table 6.

Table 6: Correlation of Histopathological Findings with Inflammatory Markers (n = 120)

Variable	Correlation Coefficient (r)	p-value
Histopathology vs CRP	0.612	<0.001
Histopathology vs ESR	0.548	<0.001
Histopathology vs TLC	0.471	<0.001

DISCUSSION

Diabetic foot ulcer is a serious complication of diabetes mellitus resulting from peripheral neuropathy, peripheral arterial disease, and impaired wound healing. It is a major cause of chronic infection, hospitalization, lower-limb amputation, and increased healthcare costs worldwide.¹⁴ Histopathological examination provides valuable information regarding tissue inflammation, necrosis, and osteomyelitis, while bacterial culture identifies the causative microorganisms responsible for infection. Inflammatory markers such as C-reactive protein, erythrocyte sedimentation rate, and total leukocyte count reflect the systemic inflammatory response and disease severity. Correlating these diagnostic parameters may improve the assessment of diabetic foot infections and facilitate timely therapeutic interventions.¹⁵ However, limited local evidence is available regarding these correlations in the Pakistani population, warranting further investigation.

The present study evaluated the correlation of histopathological findings with bacterial culture and inflammatory markers in diabetic foot ulcers. The mean age of the patients was 56.8 ± 10.9 years, with a predominance of males (63.3%). Chronic inflammation was the most common histopathological finding (43.3%), followed by acute-on-chronic inflammation (33.3%) and acute inflammation (23.3%). Tissue necrosis and granulation tissue were observed in 65.8% and 69.2% of cases, respectively, while osteomyelitis was identified in 24.2% of patients. Positive bacterial cultures were obtained in 84.2% of cases, and *Staphylococcus aureus* was the most frequently isolated organism (27.7%). Furthermore, histopathological findings showed a significant association with bacterial culture ($p=0.041$) and demonstrated significant positive correlations with CRP ($r=0.612$), ESR ($r=0.548$), and TLC ($r=0.471$) ($p<0.001$).

The histopathological findings of the present study are comparable with those reported by Jeandrot et al. (2008), who emphasized that inflammatory severity in diabetic foot ulcers is closely associated with systemic inflammatory response. They demonstrated that CRP was the most informative biomarker for differentiating infected ulcers, with a sensitivity of 72.7%, specificity of 100%, and an AUC of 0.947 when combined with procalcitonin. In contrast, leukocyte and neutrophil counts alone showed poor predictive value. Similarly, the present study found the strongest correlation between histopathological severity and CRP ($r=0.612$), followed by ESR and TLC, suggesting that CRP remains the most reliable laboratory indicator of tissue inflammation in diabetic foot ulcers.¹⁸

Cecilia et al. (2013) evaluated histopathological patterns of diabetic foot osteomyelitis and reported chronic osteomyelitis in 44.2%, acute osteomyelitis in 27.9%, chronic acute osteomyelitis in 8.5%, and fibrosis in 19.4% of patients. Bacterial cultures were positive in 87.0% of acute osteomyelitis, 83.5% of chronic osteomyelitis, and 78.6% of chronic acute osteomyelitis cases. These findings closely resemble those of the present study, where chronic inflammation was the predominant histopathological pattern (43.3%) and bacterial cultures were positive in 84.2% of patients. Likewise, culture positivity remained high across different inflammatory patterns, supporting the concept that increasing histopathological inflammation

is associated with a greater likelihood of bacterial isolation.¹⁶ Ayub et al. (2016) investigated the microbiological profile of diabetic foot infections in Pakistan and reported that aerobic bacteria accounted for 62% of isolates, while fungal or anaerobic organisms constituted 38%. They identified *Staphylococcus aureus* as the most common Gram-positive organism and *Escherichia coli* as the predominant Gram-negative isolate. The microbiological findings of the present study are in agreement, as *Staphylococcus aureus* (27.7%) was the most frequently isolated pathogen, followed by *Pseudomonas aeruginosa* (21.8%), *Escherichia coli* (18.8%), and *Klebsiella pneumoniae* (14.9%). These similarities indicate that *Staphylococcus aureus* continues to be the leading pathogen responsible for diabetic foot infections in the local population.²¹ Van Asten et al. (2017) investigated inflammatory markers in diabetic foot osteomyelitis and found significantly higher baseline procalcitonin levels among patients with osteomyelitis ($P=0.049$), whereas CRP, ESR, procalcitonin, and IL-6 significantly declined following treatment. They concluded that CRP and ESR are valuable markers for monitoring disease activity. Although procalcitonin was not assessed in the present study, the significant positive correlations observed between histopathological severity and CRP ($r=0.612$) as well as ESR ($r=0.548$) reinforce the importance of these inflammatory markers in reflecting tissue inflammation and infection severity.

Hadavand et al. (2019) demonstrated that patients with advanced diabetic foot ulcers and osteomyelitis had significantly higher CRP, ESR, and procalcitonin levels ($p<0.001$). They also reported optimal diagnostic cut-off values of 44 mg/L for CRP and 56.5 mm/hour for ESR in predicting osteomyelitis. The mean CRP (71.5 ± 34.2 mg/L) and ESR (58.9 ± 22.6 mm/hour) observed in the present study exceeded these proposed cut-off values, suggesting that the study population largely represented patients with advanced inflammatory changes. Moreover, the significant correlations between histopathological findings and inflammatory markers further support the diagnostic value of these laboratory parameters.¹⁹ Sharma et al. (2022) performed a systematic review and meta-analysis evaluating inflammatory biomarkers in diabetic foot infections and reported that CRP demonstrated the highest diagnostic accuracy for grade II diabetic foot ulcers with an AUC of 0.893, sensitivity of 77.4%, and specificity of 84.3%, whereas procalcitonin exhibited the highest diagnostic performance for grade III ulcers with an AUC of 0.844. These findings are consistent with the present study, where CRP showed the strongest correlation with histopathological severity among all inflammatory markers evaluated, highlighting its role as the most useful routine biomarker for assessing diabetic foot infections.¹⁷

More recently, Junaid et al. (2025) reported *Escherichia coli* (21.6%) and *Klebsiella pneumoniae* (10.8%) as the predominant Gram-negative pathogens, whereas methicillin-resistant *Staphylococcus aureus* and *Staphylococcus aureus* represented the most frequent Gram-positive isolates. They also observed no significant association between patient comorbidities and antimicrobial resistance patterns. Although antimicrobial susceptibility testing was beyond the scope of the present study, the bacterial spectrum showed considerable similarity, with *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* among the most frequently isolated organisms, supporting previous evidence regarding the microbiological profile of diabetic foot infections.²⁰

The findings of Yasir et al. (2026) further support the present results. They reported positive bacterial cultures in 77.9% of diabetic foot ulcer patients, male predominance (57.7%), highest disease frequency among individuals aged 52–62 years, and *Staphylococcus aureus* as the most common isolate (46.9%). Likewise, the present study demonstrated positive bacterial cultures in 84.2% of patients, male predominance of 63.3%, a mean age of 56.8 years, and *Staphylococcus aureus* as the leading bacterial isolate. The slightly higher culture positivity observed in the present study may be attributed to differences in patient selection, ulcer severity, specimen collection techniques, and microbiological processing. Overall, the findings consistently indicate that histopathological severity, bacterial infection, and systemic inflammatory response are closely interrelated in diabetic foot ulcers, emphasizing the value of an integrated diagnostic approach for optimizing patient management.²¹

STUDY LIMITATIONS

The cross-sectional design did not permit assessment of causal relationships or long-term clinical outcomes. Being a single-center study with a relatively modest sample size may have limited the generalizability of the findings. In addition, anaerobic bacterial cultures and advanced molecular diagnostic techniques were not performed, which may have underestimated the spectrum of infecting organisms.

CONCLUSION

Histopathological findings demonstrated significant correlations with bacterial culture positivity and inflammatory markers in patients with diabetic foot ulcers. The combined assessment of these parameters may improve the evaluation of infection severity and support timely clinical decision-making. Further multicenter prospective studies are recommended to validate these findings and improve management strategies for diabetic foot infections.

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Conflict of Interest: No

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