Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i8.5945

ROLE OF PATHOGENS IN DIABETIC FOOT INFECTION AND THE POTENTIAL OF IMMUNOPROTEOMICS AS A DIAGNOSTIC AND PROGNOSTIC TOOL

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Abstract

Introduction: Diabetic foot infection (DFI) stands as a prevalent and debilitating complication of diabetes mellitus, posing significant challenges to patients' quality of life and healthcare systems worldwide.

Objectives: The main objective of the study is to find the role of pathogens in diabetic foot infection (DFI) and the potential of immunoproteomics as a diagnostic and prognostic tool.

Material and methods: This prospective observational study was conducted at Rashid Latif Medical and Dental College, Lahore from June 2021 to November 2021. Data were collected from 550 diabetic patients suffering from DFI. Data were collected through a systematically designed questionnaire which included clinical evaluation, including medical history assessment, physical examination, and wound assessment.

Results: Data were collected from 550 patients from both genders. There were 60% male and 40% female. Mean age of patients was 65.09 ± 8.3 years and the mean duration of diabetes was 11.98 ± 6.51 years. 70% of the patients also suffering from hypertension, 45% Peripheral Neuropathy and 30% from Peripheral Vascular Disease. Staphylococcus aureus, both methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) strains, are the most prevalent pathogens, with MRSA exhibiting a resistance rate of 30%. Enterococcus spp., Escherichia coli, and Pseudomonas aeruginosa are also prevalent, with Escherichia coli showing notable resistance rates of 60% to ciprofloxacin and 40% to gentamicin. Elevated levels of IL-6 and TNF-alpha show positive correlations with wound size (IL-6: $r = 0.65$, $p < 0.001$; TNF-alpha: $r = 0.55$, $p = 0.005$), tissue depth involvement (IL-6: $r = 0.50$, $p = 0.002$; TNF-alpha: $r = 0.45$, $p = 0.015$), and amputation rate (IL-6: $r = 0.75$, $p < 0.001$; TNF-alpha: $r = 0.70$, $p < 0.001$).

Conclusion: It is concluded that the role of pathogens and host immune response in diabetic foot infections (DFI) is important for guiding effective treatment strategies.

Introduction

Diabetic foot infection (DFI) stands as a prevalent and debilitating complication of diabetes mellitus, posing significant challenges to patients' quality of life and healthcare systems worldwide. Characterized by microbial invasion of soft tissue and bone, DFIs frequently lead to severe complications, including amputation and mortality, particularly in cases of delayed diagnosis and inadequate treatment [1]. Pathogens play a pivotal role in the pathogenesis and progression of DFIs, with polymicrobial infections being common and challenging to manage. Early and accurate identification of the infecting pathogens is crucial for guiding appropriate antimicrobial therapy and improving patient outcomes [2].

Diabetic foot ulcer (DFU) is one of the most common problems clinicians have to deal within patients with diabetes mellitus (DM). The incidence varies between 15% and 25%, and about 1% of this population has to undergo a lower limb amputation [3]. The high incidence of severe complications and the increased rates of morbidity and mortality, prompt for early diagnosis and initiation of appropriate antibiotic treatment to improve final outcomes. Infection complicates approximately 60% of DFUs [4]. The initial soft tissue infection may spread into the bone resulting in diabetic foot osteomyelitis and thus a high risk of amputation. Osteomyelitis should be suspected in all DFU patients with clinical findings of infection and in chronic or recurrent wounds. Early identification of this clinical entity is crucial for the overall management and in order to reduce mortality [5].

DM patients with deep foot infections are 154.5 times more likely to have a leg, foot, or toe amputated compared with patients without DM. The infection is usually complicated to diabetic foot ulcer (DFU) initiated by an open wound on the foot caused by injury, ischemic, or tinea pedis [6]. The weakened immune system, impaired peripheral blood circulation, neuropathy, and peripheral vasculopathy facilitate DFI. The pathogens of DFI include aerobic bacteria such as Staphylococcus, Streptococcus, and Enterobacteriaceae, and anaerobic bacteria such as Bacteroides, Clostridium, and Peptostreptococcus, and fungi [7]. According to guidelines compiled by the Infectious Diseases Society of America, DFIs are classified into three subcategories, namely mild infections with only superficial symptoms that are limited in size and depth, moderate infections with deeper or more extensive symptoms, and severe infections accompanied by systemic signs or metabolic perturbations [8]. For the treatment of mild and moderate DFIs, oral therapy alone or followed by a short course of intravenous therapy with narrow-spectrum antibiotics is likely sufficient. Severe DFI is often associated with previously treated chronic infection, and possibly with antibiotic resistance. The initial approach for severe DFI treatment is parenteral administration of broad-spectrum antibiotics, minimally those against Staphylococcus and Streptococcus [9].

In recent years, immunoproteomics has emerged as a promising approach for the diagnosis and prognostication of infectious diseases, including DFIs. Immunoproteomics involves the comprehensive analysis of the immune response to pathogenic antigens, enabling the identification of specific biomarkers associated with infection [10]. By interrogating the host's immune repertoire, immunoproteomics offers insights into the complex interplay between pathogens and the host immune system, facilitating the development of novel diagnostic and prognostic tools for infectious diseases [11].

It is estimated that 19 to 34% of patients with T2D develop DFU in their lifetimes, and DFU is the most common cause of hospitalization and medical costs associated with diabetes. Despite high health care costs, outcomes for patients presenting with DFU infections are poor; such infections often result in lower-limb amputation, with very poor 1-, 2-, and 5-year survival rates of 80.80%, 69.01%, and 28.64%, respectively [12]. DFU is caused by a combination of peripheral sensorimotor and autonomic neuropathy, peripheral vascular disease, and minor trauma, frequently complicated by subsequent infections. Several metagenomic studies demonstrated that Staphylococcus aureus is the most common pathogen isolated from DFU infections. Furthermore, microbiome studies demonstrated that patients with T2D showed skin microbiota more frequently colonized with S. aureus and more susceptible to S. aureus infections [13].

Objectives
The main objective of the study is to find the role of pathogens in diabetic foot infection (DFI) and the potential of immunoproteomics as a diagnostic and prognostic tool.

Material and methods
This prospective observational study was conducted at Rashid Latif Medical and Dental College, Lahore from June 2021 to November 2021. Data were collected from 550 diabetic patients suffering from DFI.

Inclusion criteria
- Patients aged > 18 years
- Patients with confirm diagnosis of DFI.
- Those who are willing to participate in the study.

Exclusion criteria
- Patients with any immunodeficiency disorder.
- Patients with any inflammatory condition and using antibiotics from last two weeks.

Data collection
Data were collected from 550 diabetic patients suffering from diabetic foot infection. Data were collected through a systematically designed questionnaire which include clinical evaluation, including medical history assessment, physical examination, and wound assessment. Relevant clinical data, including demographic information, diabetes-related variables, duration of diabetes, glycemic control, and details of foot ulcer characteristics were also noted.

Microbiological Analysis: Wound swabs were collected from all patients for microbiological analysis. Standard microbiological techniques, including aerobic and anaerobic culture, were employed to identify the causative pathogens. Antimicrobial susceptibility testing was performed according to established guidelines.

Immunoproteomic Analysis: Blood samples were collected from a set of patients (n=100) for immunoproteomic analysis. Blood was centrifuged at 3000rpm for 15 mins for serum separation. Peripheral blood mononuclear cells (PBMCs) were isolated, and serum samples were used for analysis of the host immune response to DFI-associated pathogens. Enzyme-linked immunosorbent assay (ELISA) were employed to identify specific antibodies and immune markers indicative of DFI.

Data analysis
Data were analyzed using SPSS v29.0 and GraphPad prism 2021. The prevalence of different pathogens, antimicrobial resistance patterns, and immune response profiles were analyzed. P-value <0.005 were considered as significant.

Ethical consideration
All the data collected according to ethical committee of hospital and data of patients remains confidential.

Results
Data were collected from 550 patients from both genders. There were 60% male and 40% female. Mean age of patients was 65.09 ± 8.3 years and mean duration of diabetes was 11.98 ± 6.51 years. 70% of the patients also suffering from hypertension, 45% Peripheral Neuropathy and 30% from Peripheral Vascular Disease. Mean level of Hb was 11.2 ± 1.5g/dL. Demographic data is represented in table 01.

Table 01: Demographic and baseline values of patients

Characteristic	Value
Gender Male Female	 60% 40%
Age (years)	65.09 ± 8.3
Duration of Diabetes	11.98 ± 6.51
HbA1c (%)	8.5 ± 1.2
Comorbidities	
Hypertension (%)	70%
Peripheral Neuropathy (%)	45%
Peripheral Vascular Disease (%)	30%
Biochemical parameters	
Hemoglobin (g/dL)	11.2 ± 1.5
White Blood Cell Count (x10^3/µL)	11.8 ± 3.2
Neutrophil Count (%)	70.5 ± 8.7
Lymphocyte Count (%)	22.0 ± 6.3
Platelet Count (x10^3/µL)	275 ± 60
C-Reactive Protein (mg/L)	35.2 ± 15.6

Grade 1 infections, characterized as superficial, corresponded to the lowest numbers, while Grade 4, indicative of limb-threatening conditions, exhibited the highest. This underscores the correlation between infection severity and microbial burden, emphasizing the importance of early intervention and appropriate management strategies to mitigate the risk of complications in diabetic foot infections.

Table 02: DFI grade and bacterial isolation in 550 patients

Diabetic Foot Infection Grade	Number of Patients	Number of Bacteria Isolated
Grade 1 (Superficial)	100	120
Grade 2 (Deep)	200	260
Grade 3 (Extensive)	150	300
Grade 4 (Limb-threatening)	100	200

Staphylococcus aureus, both methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) strains, are the most prevalent pathogens, with MRSA exhibiting a resistance rate of 30%. Enterococcus spp., Escherichia coli, and Pseudomonas aeruginosa are also prevalent, with Escherichia coli showing notable resistance rates of 60% to ciprofloxacin and 40% to gentamicin.

Table 03: Microbiological analysis and prevalence

Pathogen	Prevalence (%)	Antimicrobial Resistance (%)
Staphylococcus aureus (MSSA)	40%	30% (Methicillin-resistant)
Enterococcus spp.	25%	
Escherichia coli	20%	60% (Ciprofloxacin), 40% (Gentamicin)
Pseudomonas aeruginosa	15%	

Elevated levels of IL-6 and TNF-alpha show positive correlations with wound size (IL-6: $r = 0.65$, $p < 0.001$; TNF-alpha: $r = 0.55$, $p = 0.005$), tissue depth involvement (IL-6: $r = 0.50$, $p = 0.002$; TNF-alpha: $r = 0.45$, $p = 0.015$), and amputation rate (IL-6: $r = 0.75$, $p < 0.001$; TNF-alpha: $r = 0.70$, $p <$

0.001). Furthermore, the presence of antibodies against DFI-associated pathogens correlates positively with treatment failure ($r = 0.60$, $p = 0.003$) and recurrent infections ($r = 0.55$, $p = 0.008$).

Table 04: Correlation of immunomarkers in DFI

Immune Marker	Clinical Outcome	Correlation Coefficient (r)	P-value
IL-6 (pg/mL)	Wound size	0.65	< 0.001
	Tissue depth	0.50	0.002
	Amputation rate	0.75	< 0.001
TNF-alpha (pg/mL)	Wound size	0.55	0.005
	Tissue depth	0.45	0.015
	Amputation rate	0.70	< 0.001
Antibodies against DFI-associated pathogens	Treatment failure	0.60	0.003
	Recurrent infections	0.55	0.008

Staphylococcus aureus, both methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) strains, exhibit notable resistance rates, with MRSA showing particularly high levels of resistance to methicillin (80%). Enterococcus spp. demonstrate moderate resistance to vancomycin (20%), while Escherichia coli and Pseudomonas aeruginosa display substantial resistance to ciprofloxacin (60%) and piperacillin/tazobactam (50%), respectively.

Table 05: Antimicrobial resistance pattern in DFI

Pathogen	Antibiotic	Resistant (%)	Intermediate (%)	Susceptible (%)
Staphylococcus aureus (MSSA)	Methicillin	30	10	60
Staphylococcus aureus (MRSA)	Methicillin	80	15	5
Enterococcus spp.	Vancomycin	20	5	75
Escherichia coli	Ciprofloxacin	60	20	20
	Gentamicin	40	30	30
Pseudomonas aeruginosa	Piperacillin/Tazobactam	50	10	40

Discussion

The study identified Staphylococcus aureus, Enterococcus spp., Escherichia coli, and Pseudomonas aeruginosa as the most prevalent pathogens in diabetic foot infections. The high prevalence of these pathogens underscores their significant role in DFI pathogenesis. Moreover, antimicrobial resistance was observed, particularly among Gram-negative bacteria, highlighting the challenge of treating DFI effectively [14]. Pathogenic bacteria and drug sensitivity spectrums vary regionally and are affected by the widespread use of antibiotics. Appropriate antibiotic selection for DFI is controversial because to date no empirical antimicrobial regimen has been shown to be superior. Thus, definitive therapy should be based on the identification of pathogens and their drug sensitivity [15]. The current study generated drug susceptibility results for a variety of bacterial pathogens isolated from patients with DFI [16].

Immunoproteomics is a powerful tool to identify immunoreactive molecules and develop candidate vaccines against pathogens. Immunoproteomics combines proteomics for the detection of immunoreactive antigens expressed during infections [17]. High throughput immunoproteomics

arrays offer a rapid, sensitive, and specific diagnosis of pathogens and their drug resistance profiles, which are otherwise difficult to culture or are multi-species infections. A viral proteome array comprising 646 viral antigens was developed by to examine the relationship between viral infections and the early onset of DM1 [18]. Immunoproteomic analysis revealed elevated levels of proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) in patients with diabetic foot infections [19]. Additionally, specific antibody responses against DFI-associated pathogens were detected, suggesting a robust immune response to infection. These findings support the potential utility of immunoproteomics as a diagnostic and prognostic tool for DFI [20].

The study demonstrated significant correlations between immune markers and clinical outcomes in diabetic foot infections. Elevated levels of IL-6 and TNF-alpha were associated with increased wound size, greater tissue depth involvement, and higher rates of amputation, indicating their potential as prognostic indicators for disease severity and progression. Furthermore, the presence of antibodies against DFI-associated pathogens was predictive of treatment failure and recurrent infections, highlighting the importance of host immune response in determining clinical outcomes [21].

The study identified concerning rates of antimicrobial resistance among pathogens isolated from diabetic foot infections. Resistance to commonly used antibiotics, particularly among Gram-negative bacteria, poses challenges in selecting appropriate antimicrobial therapy and emphasizes the need for judicious antibiotic use and antimicrobial stewardship strategies [22]. *Staphylococcus aureus* grows and secretes virulent factors in glucose-rich diabetic conditions, where insulin deficiency prevents or delays immune response. *Staphylococcus aureus* has expanded its glycolytic capacity by acquiring several additional glucose transporters. Carbohydrate transporters in *S. aureus* allow efficient uptake of carbohydrates and support anaerobic growth in inflamed tissues [23]. Eleven carbohydrate transporters have been identified in *S. aureus*, while four of them (glcA, glcB, glcC, and glcU) are strictly responsible for glucose transportation observed that in a murine model of wound infection, the inactivation of carbohydrate transporter might reduce glucose uptake and attenuate *S. aureus* growth [24].

The findings of this study have important clinical implications for the management of diabetic foot infections. Understanding the role of pathogens and host immune response in DFI pathogenesis can inform targeted therapeutic interventions and personalized treatment approaches. Additionally, addressing antimicrobial resistance through antibiotic stewardship programs and exploring alternative treatment modalities, such as immunomodulatory therapies, may improve outcomes in patients with diabetic foot infections.

Conclusion

It is concluded that the role of pathogens and host immune response in diabetic foot infections (DFI) is important for guiding effective treatment strategies. According to our study potential of immunoproteomics as a diagnostic and prognostic tool also emphasizing the concerning rates of antimicrobial resistance among DFI-associated pathogens.

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