Short communication

Bacterial and fungal profile of diabetic foot ulcer

Anjally Devasia[1], Linu Alice John[1], Saleena Ummer Velladath[1], Peralam Yegneswaran Prakash[2], Ciraj Ali Mohammad[3], Kavitha S. Shettigar[1]

[1]Department of Medical Laboratory Technology, Manipal College of Health Professions, Manipal Academy of Higher Education, Manipal, Karnataka, India
[2]Department of Microbiology, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, Karnataka, India
[3]Melaka Manipal Medical College, Manipal Academy of Higher Education, Manipal, Karnataka, India

(Received: August 2022 Revised: November 2022 Accepted: December 2022)

Corresponding author: **Kavitha S. Shettigar**. Email: kavitha.shettigar@manipal.edu

ABSTRACT

Introduction: Polymicrobial biofilm in chronic diabetic foot wounds is a major challenge for the treatment and lead to several complications, including increased morbidity, mortality, and limb loss.

Methodology: In this study, a spectrum of bacterial and fungal isolates was carried out in 66-foot ulcer samples from diabetic patients. All bacterial isolates were identified by conventional culture methods and biochemical identification tests. Filamentous fungi and yeasts were identified morphologically and by staining methods.

Results: Among 66 samples, 62 (94%) samples were culture-positive, and four samples (6%) were culture-negative by the conventional culture method. Polymicrobial growth was found in 11 samples (17%) and monomicrobial in 51 wound samples (77%). Both Gram-positive and Gram-negative bacterial species were isolated. The number of *Staphylococcus aureus* isolates was more (61%). Fungi were isolated in 4 patients, and all the isolates were identified as *Candida tropicalis*. Several clinical complications were found including hypertension (47%), osteoarthritis (6%), rheumatoid arthritis (8%), cardiovascular disease (19%), peripheral vascular disease (20%), amputation (35%), foot cramps (71%) and edema (8%).

Conclusion: Monomicrobial growth was predominant than polymicrobial growth, and *S. aureus* was the predominant pathogen isolated. Adequate treatment management of diabetic foot is needed to reduce the risk of amputation.

Keywords: Diabetic foot ulcer; bacteria; fungi; polymicrobes; biofilm.

INTRODUCTION

Foot ulcer infection is a significant consequence of diabetes. It results from the invasion of microbes into the tissue in an adequate number elevating host immune response and delaying wound healing. Diabetic foot infection (DFI) is multifactorial associated with interactions of microbes and their virulence potentials and host immune competencies (1). A sustained foot ulcer is a leading cause of developing foot infection (2,3).

The global prevalence of DFI is accounting for 25.2% to 58%. Impaired wound healing is a critical problem and affects 51% of patients with diabetes in India (4). Diabetic foot ulcers precede 84% of all diabetic-related lower-leg amputations, risk of which increases with age and duration of diabetes (5). This also presents an enormous healthcare burden and a significant reduction in the patient's overall quality of life. The rate of non-infected ulcers developing to foot infection is high and is a critical problem, and persistent infection may lead to osteomyelitis and amputation of lower extremities (3,6,7).

The healing rate of an infected foot ulcer is reported to be 44.5%, and patients require surgical interventions of revascularization and amputation (8). DFI in polymicrobial biofilm mediated and ulcer pathogens are mostly biofilm producers and drug-resistant isolates. Biofilms are present in chronic and acutely infected diabetic foot ulcers (DFUs; 9). Biofilms are extracellular matrix molecules that mediate the survival of pathogens during nutritional challenges and antibiotic treatments, which mediate persistent infection and poor wound healing (1).

Both Gram-positive and Gram-negative bacteria are associated with DFU, and most of the DFU isolates are biofilm producers (10). The fungus *Candida albicans* and bacterium *S. aureus* are the common pathogens isolated in DFU and bacterial-fungal interactions play a significant role in host-microbe interaction and wound pathogenicity (11). DFIs are polymicrobial infections associated with multidrug-resistant (MDR) isolates and biofilms producers, which is an important virulence factor and results in treatment failure (12). Previous studies estimated that 27% of candidemia are polymicrobial infections, and *S. aureus* is the third most commonly coexisting species (13). Hence, we aim to study the spectrum of bacterial and fungal isolates in DFU.

MATERIALS AND METHODS

This is a cross-sectional study conducted at diabetic foot clinics and hospitals in the Udupi district. The study was conducted after obtaining the prior approval from the District Health Officer, Udupi District, Karnataka, India. Prior written informed consent was obtained from all the patients before the collection of foot ulcer samples. The study was conducted after getting approval from the institutional ethics committee (IEC: 378/2019).

Three swabs were collected from the ulcer site for Gram staining (12), bacterial culture, and fungal culture. Swabs collected were carried to the microbiological laboratory immediately and processed for microbial profiling. Tissue slough, necrotic tissue, and wound swabs over the wounds were collected. Specimens were collected in sterile containers and transported to the microbiology laboratory. Samples were processed separately for the identification of bacteria and fungi. For bacterial isolation, samples were streaked on to the Blood agar and MacConkey agar and incubated at 37°C for 48 hrs. Bacterial isolates were identified by conventional culture methods and biochemical identification tests (13). For the isolation of fungal isolates, each swab was inoculated into two slants of Sabouraud Dextrose Agar and was incubated at 30°C and 35°C for four weeks and was monitored regularly for fungal growth. KOH (10%) wet mount preparation and Gram staining were performed for each sample. Fungal species were identified morphologically (14, 15). Filamentous fungi were determined by the slide culture method on potato dextrose agar (PDA) and by the Lactophenol Cotton Blue (LPCB) staining method. In addition, yeast species were identified to the species level by HiCrome™ Candida differential agar.

Ethical review

The study was conducted after prior written informed consent from all the participants. All the documentations were recorded in the study log. The study was approved by the institutional ethics committee (IEC number: IEC:378/2019; Date 27th January 2020). The trial has been registered at https://register.clinicaltrials.gov (CTRI/2019/09/021160).

RESULTS

A total of 66 (male, *n*=59; female, *n*=7) (Table 1) diabetic patients having foot ulcers were recruited. Several clinical complications were found in DFU patients such as hypertension (47%), osteoarthritis (6%), rheumatoid arthritis (8%), cardiovascular disease (19%), peripheral vascular disease (20%), amputation (35%), foot cramps (71%) and edema (8%).

Among 66-foot ulcer samples, 62 samples (94%) were culture-positive, and four samples (6%) were culture-negative by the conventional culture method. Polymicrobial growth was found in 11 samples (17%) and monomicrobial in 51 wound samples (77%). Both Gram-positive and Gram-negative bacterial species were isolated. The number of *S. aureus* isolates was more (61%), followed by *Pseudomonas* spp. (29%), *Proteus* spp. (7%) and *Klebsiella* spp. (3%). Fungi were isolated in 4 patients, and all the isolates were identified as *Candida tropicalis*. Molds were not isolated in our study.

Table 1: Socio-demographic, biochemical and microbiological parameters

Characteristics (*n*=66)	**Percentage (%)**
Gender	
Male	89
Female	11
Blood glucose (average mg/dl)	
RBS	244.62
FBS	165.36
PPBS	246.23
HbA1C (%)	9.68
Ulcer site	
Right	47
Left	53
Depth	
Mild	30
Moderate	56
Severe	14
Tissue type	
Epithelial	21
Granular	21
Slough	26
Necrotic	32
Clinical complications	
Hypertension	47
Osteoarthritis	6
Rheumatoid arthritis	8
Cardiovascular disease	19
Peripheral vascular disease	20
Amputation	35
Foot cramps	71
Edema	8
Microbial growth	
Monomicrobial	77
Polymicrobial	17
Culture negative	6
Bacteria	
S. aureus	61
Pseudomonas spp.	29
Proteus spp.	7
Klebsiella spp.	3
Fungi	
Candida tropicalis	100

RBS, Random Blood Sugar; FBS, Fasting Blood Sugar; PPBS, Post Prandial Blood Sugar

DISCUSSION

Diabetic foot ulcer is one of the complications of diabetes. Our study has characterized 66 diabetic foot ulcer samples using conventional microbiological and biochemical identification methods of isolation and identification of microbes. Male patients were significantly higher than females, as reported in other studies (16, 17). Microbial isolates were found in 94% of foot ulcer samples, and monomicrobial growth was predominant (77%) than polymicrobial growth (17%). Several previous studies have reported that the *Enterobacteriaceae* family members are predominantly isolated in DFU (16-19). In our study, *S. aureus* was predominantly isolated, followed by *P. aeruginosa.* Among *the Enterobacteriaceae* family, *Proteus* spp, and *Klebsiella* spp. were isolated. In previous studies from India, Gram-negative bacteria were predominantly isolated than Gram-positive bacteria (19-21). In our study, all the 66-foot ulcer samples were cultured for both bacterial and fungal isolation. Fungal growth was found only in four samples, and all the isolates were identified as *C. tropicalis*. Molds were not isolated in our study.

Fungal isolates found in DFU samples were far lower than bacterial species. This indicates the role of interaction between host factors and microbes in the wound environment. Even though polymicrobial isolates are found in DFU, few studies have reported that specific bacterial species may be involved in delayed wound progression (22). However, the polymicrobial nature of DFU is well documented by previous studies (21, 23). Further, the role of microbes and host factors on wound healing is still needed to be studied.

Limitation of the study

The study is conducted in a small sample size.

CONCLUSION

In our study, the microbiological study of foot ulcer samples from diabetic patients was performed and different species of bacterial and fungal isolates were identified. Microbial isolates were found in most of the foot ulcer samples and monomicrobial growth was predominant than polymicrobial growth. *S. aureus* was predominant isolates in our study. However, the number of fungal isolates was less compared to bacterial isolates. Adequate treatment management of diabetic foot infection is needed to control cost of care and to increase quality of life for a growing diabetic population.

ACKNOWLEDGEMENT

The authors thank Manipal Academy of Higher Education for providing the infrastructure & financial support.

CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Hurlow, J.J., Humphreys, G.J., Bowling, F.L., McBain, A. J. Diabetic foot infection: A critical complication. Int Wound J 2018;15(5):814-821.
2. Bandyk, D.F. The diabetic foot: Pathophysiology, evaluation, and treatment. Semin Vasc Surg 2018;31(2-4):43-48.
3. Jia, L., Parker, C.N., Parker, T.J., Kinnear, E.M., Derhy, P.H., Alvarado, A.M., *et al*., Incidence and risk factors for developing infection in patients presenting with uninfected diabetic foot ulcers. PLoS One 2017;12(5):e0177916.
4. Vibha, S.P., Kulkarni, M.M., Kirthinath Ballala, A.B., Kamath, A., Maiya, G.A. Community based study to assess the prevalence of diabetic foot syndrome and associated risk factors among people with diabetes mellitus. BMC Endocr Disord 2018;18(1):43.
5. Bernhardt, G.V., Shivappa, P., Shantaram, M., Jayakar, V., Lokapur, V., Pinto, J.R. Phagocytic and oxidative burst activity of neutrophils in type 2 diabetic patients with foot ulcers. Biomedicine. 2021 Dec 31;41(4):776-780.
6. Oliver, T.I., Mutluoglu, M. Diabetic foot ulcer. 2021 Aug 19. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021.
7. Pitocco, D., Spanu, T., Di Leo, M., Vitiello, R., Rizzi, A., Tartaglione, L., *et al*., Diabetic foot infections: a comprehensive overview. Eur Rev Med Pharmacol Sci. 2019;23(2 Suppl):26-37.
8. Ndosi, M., Wright-Hughes, A., Brown, S., Backhouse, M., Lipsky, B.A., Bhogal, M., *et al*., Prognosis of the infected diabetic foot ulcer: a 12-month prospective observational study. Diabet Med 2018;35(1):78-88.
9. Johani, K., Malone, M., Jensen, S., Gosbell, I., Dickson, H., Hu, H., *et al*., Microscopy visualisation confirms multi-species biofilms are ubiquitous in diabetic foot ulcers. Int Wound J 2017;14(6):1160-1169.
10. Dunyach-Remy, C., Ngba Essebe, C., Sotto, A., Lavigne, J.P. *Staphylococcus aureus* toxins and diabetic foot ulcers: Role in pathogenesis and interest in diagnosis. Toxins (Basel) 2016;8(7):209.
11. Cheong, J.Z.A., Johnson, C.J., Wan, H., Liu, A., Kernien, J.F., Gibson, A.L.F., *et al*., Priority effects dictate community structure and alter virulence of fungal-bacterial biofilms. ISME J 2021;15(7):2012-2027.
12. Banu, A., Noorul Hassan, M.M., Rajkumar, J., Srinivasa, S. Spectrum of bacteria associated with diabetic foot ulcer and biofilm formation: A prospective study. Australas Med J 2015;8(9):280-285.
13. Kean, R., Rajendran, R., Haggarty, J., Townsend, E.M., Short, B., Burgess, K.E., *et al*., *Candida albicans* mycofilms support *Staphylococcus aureus* colonization and enhances miconazole resistance in dual-species interactions. Front Microbiol 2017;8:258.
14. Gonzalez, G.M., Elizondo, M., Ayala, J. Trends in species distribution and susceptibility of bloodstream isolates of Candida collected in Monterrey, Mexico, to seven antifungal agents: results of a 3-year (2004 to 2007) surveillance study. J Clin Microbiol 2008;46(9):2902-2905.
15. Silva, A.P., Miranda, I.M., Lisboa, C., Pina-Vaz, C., Rodrigues, A.G. Prevalence, distribution, and antifungal susceptibility profiles of Candida parapsilosis, C. orthopsilosis, and C. metapsilosis in a tertiary care hospital. J Clin Microbiol 2009;47(8):2392-2397.
16. Murali, T.S., Kavitha, S., Spoorthi, J., Bhat, D.V., Prasad, A.S.B., Upton, Z., *et al*., Characteristics of microbial drug resistance and its correlates in chronic diabetic foot ulcer infections. J Med Microbiol 2014;63(Pt 10):1377-1385.
17. Fatima, H.K., Suhad, H.M. Microbiological profile, antibiogram, and risk factors of patients with diabetic foot

infections: A systemic metaanalysis. Biomed Biotechnol Res J 2021;5:235-244.
18. Citron, D.M., Goldstein, E.J., Merriam, C.V., Lipsky, B.A., Abramson, M.A. Bacteriology of moderate-to-severe diabetic foot infections and in vitro activity of antimicrobial agents. J Clin Microbiol 2007;45(9):2819-2828.
19. Zubair, M., Malik, A., Ahmad, J. Clinico-microbiological study and antimicrobial drug resistance profile of diabetic foot infections in North India [published correction appears in Foot (Edinb). 2011;21(4):209-210.
20. Gadepalli, R., Dhawan, B., Sreenivas, V., Kapil, A., Ammini, A.C., Chaudhry, R. A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care 2006;29(8):1727-1732.
21. Ramakant, P., Verma, A.K., Misra, R., Prasad, K.N., Chand, G., Mishra, A., *et al*., Changing microbiological profile of pathogenic bacteria in diabetic foot infections: time for a rethink on which empirical therapy to choose? Diabetologia 2011;54(1):58-64.
22. Trengove, N.J., Stacey, M.C., McGechie, D.F., Mata, S. Qualitative bacteriology and leg ulcer healing. J Wound Care 1996;5(6):277-280.
23. Kalan, L.R., Meisel, J.S., Loesche, M.A., Horwinski, J., Soaita, I., Chen, X., *et al*., Strain- and species-level variation in the microbiome of diabetic wounds is associated with clinical outcomes and therapeutic efficacy. Cell Host Microbe 2019;25(5):641-655.