Original article

Microbiological and clinical characteristics of diabetic foot infections in northern India

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Abstract

Introduction: India has the largest diabetic population of 50.8 million that could reach an epidemic proportion by 2030. Diabetic foot infection is one of the dreaded complications of diabetes. Only a few studies that focus on patterns of diabetic foot infection in our region, where diabetic foot care is inadequate, are available. This study evaluated microbial and clinical characteristics of diabetic foot infections that will be helpful in taking appropriate measures for their management.

Methodology: In this prospective study conducted during 2008-2009, sixty-two diabetic foot patients underwent detailed history, clinical examination, and laboratory investigations including parameters of systemic infections. Microbial culture and sensitivity were performed at the time of presentation.

Results: Among 62 cases, 43.5% had mono-microbial infection, 35.5% had poly-microbial infections, and 21% had sterile culture. Among 82 bacteria isolated, 68% were Gram negative and 32% were Gram positive. Leukocyte counts were higher (16928±9642 versus 14593±6687 cells/mm^3) and haemoglobin (7.9±2.4 versus 9.2±2.2 mg/dl) lower in poly-microbial compared to mono-microbial infections. Haemoglobin counts were lower and leukocyte counts higher in Gram-negative compared to Gram-positive infections. Patients with sterile cultures also had clinical evidence of persistent infection. *Escherichia coli* were the most common isolate and piperacillin/tazobactam showed highest sensitivity.

Conclusions: Gram-negative bacteria were most prevalent in diabetic foot infection. It is not uncommon to have culture reports negative despite clinical evidence of infection. This study suggests that piperacillin/tazobactam should be the treatment of choice on an empirical basis prior to a definitive bacteriological study and in cases with negative culture reports.

Key words: diabetic foot, infection, microbiology, antibiotic sensitivity

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Introduction

India has a diabetic population of about 50.8 million, which is expected to increase to 87 million by 2030 [1]. Studies report that 15% of all diabetic patients develop a foot ulcer at some point in their lifetime and around 28% of them may require some form of amputation [2,3]. Foot infections account for 20% of hospitalization of diabetic patients yearly [4]. The magnitude of the problem becomes worse in regions where foot care is inadequate [5]. Infection worsens the wound condition, delays the healing mechanism and, if appropriate measures are not taken in time, could lead to systemic infection, septicaemia, amputation or even death. It is always necessary to evaluate different microorganisms infecting the wound on a routine basis in addition to administering regular glycemic control, wound care, surgical debridement, pressure-offloading, and maintaining adequate blood supply [6].

Patterns of microbes infecting diabetic foot wounds have been studied widely [7-10]. Bacterial profiles have been reported from various regions indicating area-specific studies to be conducted for assessing the problem of DFI (diabetic foot infection) and instituting effective treatment. Within the same context, we designed the present study to evaluate the microbial and clinical characteristics of diabetic foot infection in our population.

Methodology

A prospective study was performed at University Hospital in Varanasi, India. Diabetic patients with foot ulcers were recruited in the study during the year 2008 – 2009 after obtaining written informed consent. The study was approved by the institute ethics committee. All subjects underwent detailed history and clinical examination. Demographical data that included age, sex, duration of diabetes, duration of diabetic foot, location of foot ulcer, and Wagner's grade were recorded for every case. Blood was collected for clinical investigations, such as complete blood count (haemoglobin-Hb; total leukocyte count-TLC; differential leukocyte- DLC), and glycosylated haemoglobin (HbA_{1C}). Tissue/swab samples were collected for microbial culture and antibiotic sensitivity tests. To eliminate the possibility of isolating colonizing bacteria, superficial ulcers of Wagner's grade 1 were excluded from the study. After rinsing the wound area with saline and debriding the dead tissue, swab/tissue samples were collected aseptically from the wound site using a sterilized punch biopsy needle (6 mm) under local anaesthesia and placed in a sterile vial containing phosphate buffered saline. Photographs of the wound area were also taken to document depth, ischemic changes, and characteristics of the diabetic foot wound. All the above tests were performed on the day of enrolment.

Bacterial culture, isolation and identification

The tissue sample was homogenized thoroughly in the same vial in which it was collected. A loop full (one micro litre) of the homogenized samples was used to streak on the nutrient agar (Himedia Laboratories, Mumbai, India) plate and incubated for 24 hours at 37^{o}C. Pure cultures of each bacterial isolate were obtained by repeated streaking on nutrient agar plates. Identification of isolated bacteria was performed based on Gram staining and biochemical characteristics using standard methods.

Antibiotic sensitivity test

Antibiotic **s**ensitivity tests for the isolated bacteria were performed by disc diffusion method [11] against commonly used antibiotics (Himedia Laboratories) for aminoglycosides [amikacin, gentamycin], the beta-lactam group [ampicillin, cloxacillin], macrolides [azithromycin] cephalosporins [ceftriaxone, cefotaxime, cefoperazone], quinolone [levofloxacin, ciprofloxacin] penicillin combinations [piperacillin+tazobactam], *etc.* The isolate was scored as resistant or susceptible on the basis of CLSI guidelines [12].

Statistical analysis

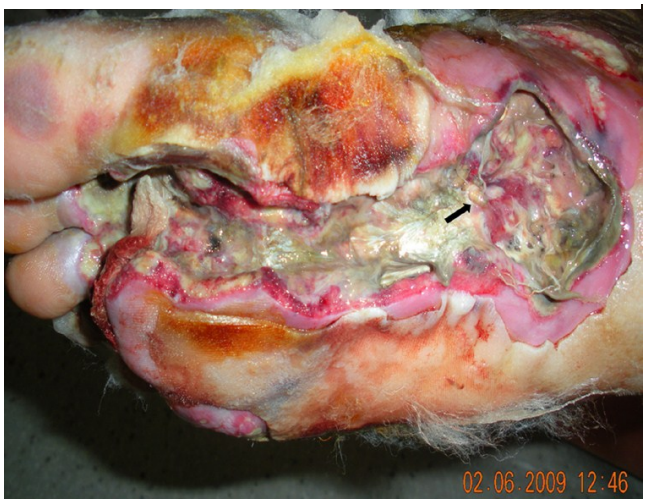
Data was represented as mean (± SD) and analyzed using SPSS 16.0 (SPSS IBM, Chicago, USA). Independent "t" test and non-parametric Mann Whitney test were applied to compare the clinical characteristics of diabetic foot patients.

Results

Sixty-two diabetic foot cases (male: female ratio = 42:20) were included in this study. The mean age of cases was 52.4 (± 11.6) years. The duration of diabetes ranged from less than a year to 20 years with a mean duration of 5.9 (± 5.5) years. The duration of diabetic foot ulcer varied from five days to one year and the cases enrolled were of Wagner's grade 2 to 4. Among 62 cases, 27 (43.5%) had mono-microbial infection, 22 (35.5%) had poly-microbial infection, and 13 (21%) had sterile culture. Altogether 82 bacteria were isolated from 49 cases. Among 82 bacterial isolates, 56 (68.3%) were Gram negative while 26 (31.7%) were Gram-positive bacteria. *Escherichia coli* was the most common pathogen isolated followed by *Staphylococcus aureus*. Other commonly isolated bacteria were *Pseudomonas aeruginosa, Streptococci, Proteus mirabilis, Citrobacter* sp.*, Proteus vulgaris, Klebsiella pneumoniae, Bacillus* sp.*, Morganella* sp.*, Acinetobacter* sp.*, Enterococcus faecalis, Klebsiella oxytoca, Enterobacter aerogenes,* Coagulase –ve Staph*, Pneumococcus, Enterococci.* Co-infection with *Candida* spp. was also found in one case with Gram-negative infection (*E. coli*). In another case with a sterile culture report, the wound was foul smelling and full of maggots (Figure 1). Gram-negative infection was most common (74%) in mono-microbial infections, whereas both Gram-positive and Gram-negative were high (63.6%) in cases with poly-microbial infection.

Diabetic foot patients with poly-microbial infection had a comparatively higher total leukocyte count (16,928 ± 9,642 versus 14,593 ± 6,687: p = 0.4) and significantly lower haemoglobin (7.9 ± 2.4 versus 9.2 ± 2.2; p = 0.02) than the mono-microbial infections, whereas HbA_1C in both the groups was similar (9.9% versus 9.5%; p = 0.1). Patients infected with Gram-negative bacteria also had significantly lower Hb (8.5 ± 1.9 versus 11.1 ± 2.2; p = 0.01), higher TLC (16280 ± 6806 versus 9771 ± 3243; p = 0.03), and a higher percentage of neutrophils (77 versus 67; p = 0.03) than those infected with Gram-positive bacteria. Patients infected with both Gram-positive and Gram-negative bacteria

Figure 1. Infected diabetic foot wound (Wagner's grade 4) with maggots.



had significantly lower Hb (7.6 ± 3.2 versus 11.1 ± 2.2; p = 0.003) compared to those infected with only Gram-positive microbes; however, the difference was insignificant when compared to patients infected with only Gram-negative bacteria. On the other hand, diabetic foot patients with sterile culture reports were clinically found to have some evidence of persistent infection. Their wounds were foul-smelling and they had raised TLC (12233 ± 3469 cells/mm^3), lower mean hemoglobin (9.5 ± 1.8 mg/dl), and HbA_{1C} of 9.5%; these reports were similar to those in patients with positive cultures.

Isolated bacteria showed differential sensitivity patterns against commonly used antibiotics. The majority of the isolates were resistant to several antibiotics that are usually prescribed on an empirical basis. Antibiotic sensitivity of the isolated microbes showed highest sensitivity for piperacillin/tazobactum, followed by amikacin, gentamycin, levofloxacin, and azithromycin.

Discussion

Diabetic foot ulcers are more prone to bacterial infections that spread rapidly, leading to irreversible tissue damage [13,14]. Complications usually begin with an unrecognized foot ulcer in a patient with an insensate foot which gets infected, leading to significant morbidity and lower extremity amputations [15]. Patterns of microbial infection are not consistent in patients with diabetic foot infections and therefore repeated evaluation of microbial characteristics and their antibiotic sensitivity is necessary for selection of appropriate antibiotics. Progression of infection in diabetic foot is a result of suppressed immune status, delayed diagnosis, underestimation of extent of infection, or suboptimal (if not inappropriate) antimicrobial therapy [16].

We observed that Gram-negative infections were more common in the studied population. In previous reports, researchers have shown the predominance of Gram-positive infections in their regions [17]. Similar observations were reported in another study conducted on a southern Indian population [7]. Diabetic foot is known for poly-microbial infections [7-10,18,19], but we observed predominantly mono-microbial infections and our finding was in accordance with those of another similar study by Dhanasekaran *et al.* [8].

Clinical characteristics such as higher TLC and lower haemoglobin levels in poly-microbial in comparison with mono-microbial infections showed that infection with multiple microorganisms not only contributed to deterioration of the wound condition locally, but also led to systemic involvement. The glycemic status of patients does not influence the microbial pattern of the infected foot wound, as evident from the HbA_{1C} value (9.9% in Gram negative versus 9.5% in Gram positive). Lower Hb and higher TLC suggest the possibility of either Gram-negative organisms alone or poly-microbial infection with at least one Gram-negative microorganism. Deteriorating wound condition, raised TLC, low haemoglobin, and high HbA_{1C}, even in culture-sterile diabetic foot patients, does not preclude presence of systemic infection. The sterile culture in such cases could be due to the use of inappropriate multiple systemic antibiotics or the application of topical antibiotics to the wound area as primary care.

We recommend the use of molecular tools for diagnosis of bacterial infection only in such situations where suspicion of infection is high despite the negative culture. Application of advanced techniques, such as rDNA PCR, ERIC PCR, *etc.*, to evaluate the infection status and bacterial diversity of the isolates in diabetic foot wounds was suggested in the literature [18,20,21]. Measurement of inflammatory markers has also been used for distinguishing infected and non-infected foot ulcers in subgroups of diabetic patients [22]. However, the positive results of culture sensitivity will always receive priority over the molecular study results for the selection of antibiotics. If we have knowledge regarding the characteristics of infection, *i.e.*, the type of bacteria commonly found and the clinical evidence of infection, the antibiotic selection can be close to appropriate, even if the culture reports are not available at the time of initiation of antibiotic therapy.

In conclusion, prevalence of Gram-negative infection was higher in diabetic foot patients from our region. In cases of poly-microbial infection, coexistence of Gram-negative and Gram-positive microorganisms was more common. Piperacillin-tazobactum showed the highest sensitivity and it may be started empirically based on the clinical characteristics of infection, and can be changed subsequent to learning the results from a definitive bacteriological study. Sometimes culture reports are negative despite the deteriorating condition of the wound and other clinical findings. In such cases, application of molecular techniques may help to identify microorganisms in the diabetic foot wound and to choose suitable antibiotics against them.

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