Diabetes mellitus. 2015;18(2):79-83

Клинико-микробиологическое исследование тканей диабетической стопы после ампутации в специализированной клинической больнице в Северной Индии

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У пациентов с сахарным диабетом (СД) на 80% выше риск развития флегмоны подкожно-жировой клетчатки, в 4 раза – риск остеомиелита и в 2 раза – риск сепсиса и летального исхода.

Цель. *Настоящее исследование было проведено для оценки клинических и микробиологических характеристик микроорганизмов, выделенных из тканей 25 пациентов с СД после ампутации конечностей.*

Материалы и методы. *В данном исследовании 25 пациентам с СД были выполнены ампутации конечностей. Язвы конечностей были классифицированы по Вагнеру, выполнена окраска материала по Граму. Также проводилось исследование влажных препаратов, фиксированных гидроксидом калия. Были проведены посевы на кровяной агар, агар МакКонки, декстрозный агар Сабуро и в бульон с сердечно-мозговой вытяжкой, и оценен рост микроорганизмов. Проводилось изучение гистологических срезов с использованием специальных красителей.*

Результаты. *В исследовании участвовали 25 пациентов (16 – мужчин, 9 – женщин). Возраст пациентов составлял от 30 до 90 лет (средний возраст: 58±10,91 лет). У большинства пациентов были язвы 3-й степени по Вагнеру. Остеомиелит был диагностирован в 13 (52%) случаях: острая форма в 2 (8%) случаях, хроническая — в 3 (12%) случаях, обострение хронического остеомиелита — в 8 (32%) случаях. По результатам посевов в большинстве случаев выделялся Proteus mirabilis; на втором месте — Escherichia coli. В 20 случаях было выделено более одного вида бактерий. Грибы Candida были выделены в 8 случаях; Trichosporon — в 2 случаях, а Fusarium — в 1 случае. При гистологическом исследовании Candida были обнаружены у 3 пациентов, а Trichosporon — у 1 пациента. В 80% случаев остеомиелита наблюдалась полимикробная инфекция.*

Заключение. *Выделение возбудителя инфекции помогает назначить подходящую схему антибиотикотерапии, что снижает частоту множественной лекарственной устойчивости, осложнений и ампутаций конечностей у пациентов, страдающих СД.*

Ключевые слова: *сахарный диабет; ампутация; инфекции; бактериологический посев*

Clinico-microbiological study of diabetic limb amputations in a tertiary care hospital in North India

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The diabetic population faces 80% increased risk of cellulitis, 4-fold increased risk of osteomyelitis and 2-fold risk of both sepsis and death caused by infections.

Study objectives: The present study was carried out to assess the clinical aspects and microbiological profile of organisms isolated from 25 patients undergoing diabetic limb amputations.

Materials and Methods. *In 25 diabetes persons who underwent limb amputation, grading of ulcers was done according to Wagner system. Material was stained with Gram stain. Potassium hydroxide wet mounts were also studied. Culture was done in blood agar, MacConkey agar, Sabouraud dextrose agar tube slants and brain heart infusion broth and examined for growth. The histopathology sections were also studied and special stains were done.*

Results. *Of 25 cases, 16 were males and 9 were females. The age ranged from 30 to 90 years (mean: 58±10.91). Majority of ulcers were grade 3. Osteomyelitis was seen in 13 (52%) cases; acute in 2 (8%), chronic in 3 (12%) and acute exacerbation of chronic osteomyelitis in 8 (32%) cases. On culture Proteus mirabilis was isolated in majority of cases followed by Escherichia coli. In 20 cases more than one bacterium were isolated. Candida was cultured in 8 cases followed by Trichosporon in 2 and Fusarium in one case. On histopathology Candida was seen in 3 cases, while one case showed spores of Trichosporon. 80% cases with osteomyelitis had polymicrobial infection.*

Conclusions. *The isolation of etiologic agent helps in administering appropriate antibiotic regimens, thus reducing the problem of multidrug resistance, morbidity and surgical limb amputations in patients suffering from diabetes mellitus.*

Key words: *diabetes; amputation; infection; culture*

DOI: 10.14341/DM2015279-83

Diabetes mellitus. 2015;18(2):79-83

Introduction

The growing diabetic population is one of the most important issues facing health-care providers today. The prevalence of diabetes mellitus has increased by more than 60% from 1990-2001 and it is estimated that the number will further increase by 165% by 2050 [1]. Compared to non-diabetics, the diabetics are at 80% increased risk of cellulitis (infection of skin and soft tissue), 4-fold increased risk of osteomyelitis and 2-fold risk of both sepsis and death caused by infections [2, 3].

Patients with diabetes mellitus have impaired leucocyte function [4]. Metabolic abnormalities of diabetes mellitus lead to inadequate migration of neutrophils and macrophages to the wound along with reduced chemotaxis [5]. The causative pathogens are varied and can range from aerobic organisms such as *Staphylococcus aureus*, group B *Streptococci*, *Enterococcus*, *Staphylococcus* (coagulase-negative), *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter*, *Citrobacter* and *Serratia*. In addition, anaerobic organisms such as *Bacteriodes*, *Peptococcus* and *Peptostreptococcus* are often identified [6]. Fungal pathogens are occasionally seen in diabetic foot ulcers, among these *Candida* species predominate [7].

Aim

The present study was undertaken due to the paucity of data on clinico-microbiological aspects of diabetic foot ulcers in Indian set-up. The aim of this study was to study the clinical aspects and microbiological profile of organisms isolated from patients undergoing limb amputations consequent upon complicated diabetes mellitus.

Materials and methods

The study was done in the Department of Pathology in collaboration with Departments of General Surgery and Microbiology on 25 patients who underwent diabetic limb amputation in a tertiary care teaching hospital. The study protocol was approved by the Institutional Ethics Committee and informed consent was obtained from all patients included in the study.

A thorough clinical assessment of sensory symptoms like burning or shooting pain, electrical or sharp sensations, numbness, etc. and clinical examination using simple hand held devices was done. For evaluation of peripheral neuropathy 10 g monofilament pressure testing, vibration sensation testing with a 128 Hz tuning fork and ankle reflex testing with a tendon hammer were done.

Material from the ulcer/necrotic area was taken by the surgeon after brief cleaning of the ulcer surface with saline and sterile gauge. Samples of exudates were taken by rubbing the surface with a sterile cotton swab and deep samples by using a 10-15 number scalpel blade and samples were collected in sterile swabs, vials or culture tubes. One part was stained with Gram staining and examined microscopically. The other part was inoculated in blood agar and MacConkey agar [8].

A small amount of specimen was examined after partial digestion with 10-20% potassium hydroxide (KOH). Sample inoculation in Sabouraud dextrose agar (SDA) tube slants was done in duplicate with incubation at 37°C and 22°C. The sample was also inoculated in brain heart infusion broth with incubation at 37°C. Culture was examined for growth daily for first week and twice a week for the next 3 weeks [9]. For histopathology amputated specimens were put in 10% buffered formalin, 3-5 micron sections were taken and stained with hematoxylin and eosin. Special stains like periodic acid Schiff (PAS), Gram and Grocott were done.

Results

Out of 25 cases, 16 were males and 9 were females. The age ranged from 30 to 90 years with a mean of 58±10.91 years. Most of the patients (60%) were in fifth and sixth decade of life. On clinical examination 23 cases showed absent pulses in lower limbs. 14 cases showed loss of sensations. Of 25 cases, neuropathic ulcers were in 14 (56%) cases while neuroischemic ulcers were present in 11 (44%) cases. Below knee amputation was done in 20 cases and above knee amputation in 2 cases. In 3 cases tarsometatarsal amputation was done. Grading of ulcers was done according to Wagner system (Table 1) (Fig. 1).

Osteomyelitis was seen in 13 (52%) cases; acute in 2 (8%) (Fig. 2), chronic in 3 (12%) and acute exacerbation of chronic osteomyelitis in 8 (32%) cases.

On culture *Proteus mirabilis* was isolated in majority of cases followed by E. coli. In 20 cases more than one bacterium were isolated (Table 2).

For fungi KOH wet mount from pus from necrotic area was done (Fig. 3a,b) and in 22 cases there were no fungi.

The cultures were examined for growth (Fig. 4a-c). *Candida* was cultured in 8 cases followed by *Trichosporon* in 2 and *Fusarium* in one case. On histopathology with PAS stain budding yeast with pseudohyphae of *Candida* were seen in 3 cases, while one case showed spores of *Trichosporon*. There was absence of osteomyelitis in *Staphylococcus aureus* infection. Eighty percent cases with osteomyelitis had polymicrobial infection. Osteomyelitis was seen in 3 (50%) cases with *Pseudomonas*, 5 (45%) cases with *Enterobacter*iaceae and 1 (33%) case with *Enterococcus* infection.

Discussion

Diabetes mellitus is a metabolic disorder in which there is abnormality in the metabolism of glucose due to qualitative and quantitative deficiency of insulin [10]. The number of patients with diabetes mellitus is increasing by epidemic proportions and the disease leads to end-organ damage due to years of hyperglycemia, which in turn results in a major burden on health care providers.

The age range of patients undergoing diabetic limb amputations in the present study was 30 to 90 years (median 59 years). The mean age was 58.6±10.91 years. Male predominance was seen in patients with diabetic foot lesions

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Fig. 1. Below knee amputation specimen of diabetic foot showing Wagner grade 3 ulcer

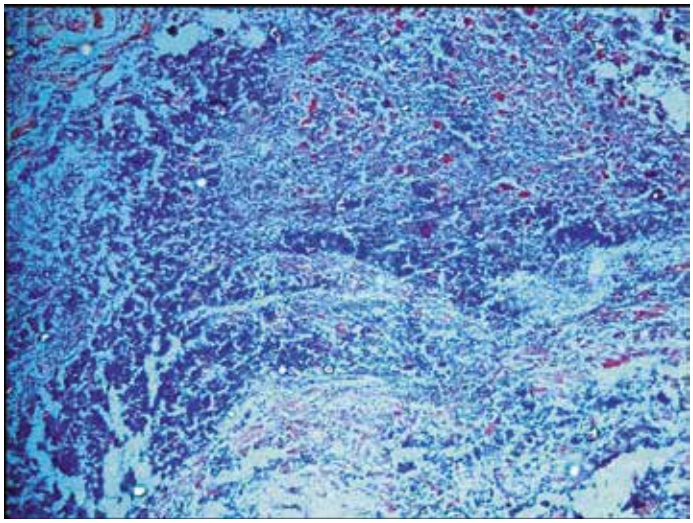

Fig. 2. Bacterial colonies seen in diabetic foot ulcer (H&E, x400).

Table 1

Grading of ulcers in diabetic limb amputations (n=25)

Grade	Characteristic features	Number of cases (%)
0	No ulcer but high risk foot	0 (0)
1	Superficial ulcer	1 (4)
2	Deep ulcer without bone involvement	8 (32)
3	Abscess with bone involvement	13 (52)
4	Localized gangrene	2 (8)
5	Gangrene involving whole foot	1 (4)

in our study, with male to female ratio of 1.7:1. The interval between the occurrence of foot lesions and seeking hospital check up ranged from 3 days to 6 months with a mean interval of 7 weeks.

In the present study maximum number of patients had Wagner grade 3 ulcers in 13 (52%) cases followed by grade 2 ulcers in 8 (32%) cases. The high incidence of deep foot ulcers in diabetes mellitus patients in our study could be because of self-medication, ignorance and poverty with a consequent delay in reporting to the hospital.

A study by Sharma et al [11] shows *Staphylococcus aureus* (38.4%), *Pseudomonas* aeruginosa (17.5%), *Proteus mirabilis* (14%) and E. coli (9.3%) as the most frequent organisms isolated in diabetic foot infections. Western literature shows aerobic Gram-positive cocci as the predominant microorganisms that colonize and acutely infect the breaks in the skin [12-18]. *Staphylococcus aureus* and the β-hemolytic *Streptococci* (Groups A, C and G but especially group B) are the most commonly isolated pathogens. Chronic wounds develop a more complex colonizing flora, including Enterococci, *Enterobacter*iaceae, obligate anaerobes, *Pseudomonas* aeruginosa and sometimes other non-fermentative Gram-negative rods [19].

Studies from India in contrast show that Gram-negative aerobic bacteria are more frequently isolated [20-22]. The major infective organisms in diabetic foot ulcers in Indian patients appear to be different. The ratio of Gram-positive aerobes to Gram-negative aerobes was 2:3, which in reversal to that reported earlier [22]. The differences in the age-sex composition, ulcer grades, and different study populations could be the plausible reasons for such variations. In the present study Gram-negative aerobes were more frequently isolated than the Gram-positive aerobes. *Proteus mirabilis* 12 cases (48%) and Escherichia coli 11 cases (44%) were the commonest bacterial species.

Amongst the isolated fungi, *Candida* was seen in 8 cases (32%), *Trichosporon* in 2 cases (8%) and *Fusarium* in one case (4%) in the current study. However, a higher percent-

Table 2

Microorganisms isolated in diabetic limb amputations (n=25)

Bacteria isolated, number of cases (%)	Fungus isolated (KOH mount), number of cases (%)
Proteus mirabilis,12 (48)	Budding yeasts with pseudohyphae of Candida, 2 (8)
Escherichia coli,11 (44)	Broad hyphae with right angle branching suggestive of mucormycosis, 1 (4)
Pseudomonas aeruginosa, 6 (24)	
Acinetobacter species, 5 (20)	No fungal elements, 22 (88)
Klebsiella oxytoca, 5 (20)	Fungus isolated on culture, number of cases (%)
Enterococcus, 3 (12)	Candida species, 8 (32)
Proteus vulgaris, 2 (8)	Trichosporon species, 2 (8)
Staphylococcus aureus, 1 (4)	Fusarium, 1 (4)
Citrobacter koseri, 1 (4)	Sterile, 14 (56)

KOH: Potassium hydroxide

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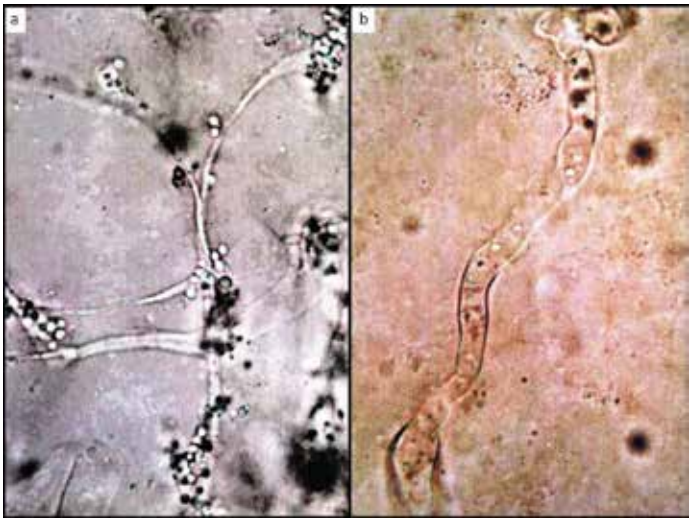

Fig. 3. KOH wet mounts: a Budding yeasts with pseudohyphae of Candida (x400). b Broad hypha of Mucormycetes (x400).

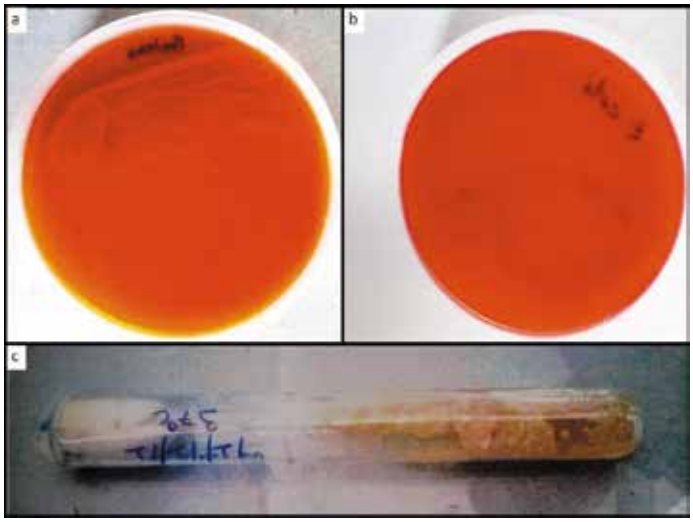

Fig. 4. Microbial growth on culture: a) Plate of MacConkey agar with growth of *Proteus;* b) Plate of blood agar showing growth of *Escherichia coli;* c) Tube with Sabouraud dextrose agar slant showing yeast like colonies of *Candida*.

age of pathogenic yeasts in 77% cases with *Candida* species being predominant were observed in another study [7]. *Candida* species were preponderant among fungal isolates in a study by Chincholikar and Pal [23].

A substantial number of cases showed osteomyelitis in the present study (13 cases; 52%). More cases of chronic than acute osteomyelitis were seen which is explained by larger time gap between the causation of foot lesions and presentation to the hospital. When microbial etiologic correlation was done, majority (80%) were polymicrobial. Amongst the single isolate, *Pseudomonas* caused osteomyelitis in half of the cases having infection. The most common causative pathogen is *Staphylococcus aureus* in diabetic foot osteomyelitis [24]. Other aerobic Gram-positive cocci followed by various aerobic Gram-negative bacilli are next in the frequency [25]. *Pseudomonas* infections usually occur in patients who have been soaking their foot or having a puncture wound particularly of the calcaneus and in those wearing shoes with rubber soles.

Conclusions

There was preponderance of Gram-negative aerobic bacteria among microbial isolates in diabetic foot ulcers in our patients. Larger multi-institute and multidisciplinary studies are required to further explore the varied microbiologic spectrum of isolates. The results of the present study are useful in studying the clinical and microbiologic variability of organisms in diabetic limb amputations in our population, considering the scarcity of studies on the subject in Indian context. The isolation of etiologic agent helps in administering appropriate antibiotic regimens. This helps in reducing the problem of multidrug resistance, morbidity and surgical limb amputations in patients suffering from diabetes mellitus.

Disclosure

Authors declare no conflict of interests regarding the article.

Authors' contributions

RSP conceived the project idea and designed the study. VJ and RK collected, analyzed and interpreted the data. RSP, RK and VJ were involved in drafting of the manuscript. RSP, AKA and JC reviewed the manuscript for important intellectual content. All authors read the manuscript draft and approved the final copy for submission.

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