

Original article:

Comparison of bacterial etiology of non-healing ulcers in diabetic and non-diabetic patients.

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Abstract:

Introduction: Non-healing ulcers of foot ,particularly in diabetic patients, cause a major medical and economic problem. Diabetes mellitus is not the only cause of non-healing ulcers. There are very few studies where the etiology of non-healing ulcers from non-diabetic patients is studied. We wanted to study, whether there is any difference between such ulcers from diabetic individuals and non-diabetic individuals.

Materials & Methods: A total of 86 patients with the non-healing foot ulcers were included in the study. The duration of ulceration was minimum 3-4 weeks which did not respond to conventional therapies. Fifty of them were Diabetic (BSL>200) while other thirty six were non diabetic patients (BSL<200). All the isolates were identified using standard microbiological techniques. Antibiotic sensitivity was tested by Kirby-Bauer disc diffusion technique.

Results: out of total 86 samples, growth was obtained in 74 samples (86%). When diabetic and non-diabetic groups were compared, there was a striking difference in the etiology. No growth was seen in 8% of diabetic cases and 22% of non-diabetics. Polymicrobial infections were predominant in diabetic group (52%) than non-diabetic group (25%). Staphylococcus aureus dominated the non-diabetic group as a single isolate from 16 cases (43.2%). In diabetic group, Gram negative bacilli were the main cause on 44 occasions (61%).

Conclusion: From the present study we can conclude that the etiology of non-healing ulcers in diabetic patients is different than that of the non-diabetic patients. Giving targeted treatment after knowing the causative agent and its drug sensitivity pattern is the best way to treat non-healing ulcers.

Key words: Non-healing ulcers, Diabetic patients, non-diabetic patients

Introduction:

Non-healing ulcers of foot are common and represent a serious health problem. Particularly, foot ulcers in patients with diabetes cause a major medical and economic problem. This is the leading cause of hospitalization for patient with diabetes mellitus [1]. The diabetic population of India is likely to increase to 87 million by 2030 [2]. That is when the problem of non-healing foot ulcers will be still more important. Presence of bacteria in the ulcers affects and delays the healing process. It also

complicates the pathological picture of diabetic foot [3]. Such diabetic foot ulcers are generally treated empirically, but a directed therapy with a known causative organism can improve the outcome [4]. Diabetes mellitus is not the only cause of non-healing ulcers. There are some other conditions like peripheral vascular disease, peripheral neuropathy, coronary artery disease, paraplegia etc. [5]. There are very few studies where the etiology of non-healing ulcers from non-diabetic patients is studied. Majority of the published data is about etiology of ulcers in

diabetic patients [4, 6, 7, 8]. We wanted to study, whether there is any difference between such ulcers from diabetic individuals and non-diabetic individuals. Therefore the study was conducted with following aims and objectives-

Aims & Objectives:

1. To study the bacterial etiological agents involved in causation of non-healing ulcers.
2. To compare the etiology of diabetic ulcers and non-diabetic ulcers.
3. To check the antibiotic sensitivity pattern of the isolates.

Materials & Methods: A total of 86 patients who visited the surgery OPD with the complains of non-healing chronic ulcers were included in the study. All the patients had foot ulcers. The duration of ulceration was minimum 3-4 weeks which did not respond to conventional therapies. Fifty of them were suffering from Diabetes mellitus (BSL>200) while other thirty six were non diabetic patients (BSL<200). 22 of the total patients with persistent infection revisited the OPD and samples were repeated from these cases. Duration between two samples collected was minimum 1 month.

Inclusion criteria: Only patients with superficial ulcers were included in the study. In diabetic group Wagner grades 0,I and II were included.

Exclusion criteria: Patients having deep wounds were excluded i.e. Wagner grades III and above.

From each patient, two swabs were collected from the superficial skin ulcers. Proper precautions were taken to avoid contamination by normal flora. The swabs were transported immediately to the laboratory. Primary smear examination was done by Gram’s staining. All the samples were then inoculated on Blood agar and MacConkey’s agar for aerobic culture and for anaerobes thioglycolate broth and RCM were used.

All the isolates were identified using standard microbiological techniques. Antibiotic sensitivity was tested by Kirby-Bauerdisc diffusion technique [9]. Anaerobes were broadly identified using following criteria- Growth in Thioglycolate broth & RCM, inhibition of growth aerobically and Gram staining.

Results:

The age of patients ranged from 31 years to 80 years in diabetics (mean being 55). In non-diabetics, it was 12 years to 61 years (mean being 36). In diabetics maximum patients were in the age group of 61 to 70 while in the non-diabetics it was 41-50. Males dominated in both the groups.

Sex distribution

	Diabetic group	Non-diabetic group
Male	34	28
Female	16	08
Total	50	36

Out of total 86 samples, growth was obtained in 74 samples i.e. 86% cases. Polymicrobial growth was seen in 35 cases (41%). When diabetic and non-diabetic groups were compared, there was a striking difference in the etiology. No growth was seen only in 8% of diabetic cases but in non-diabetics in 22%

cases growth was absent. Polymicrobial infections were predominant in diabetic group (52%). From non-diabetic group 25% cases yielded polymicrobial growth.

Growth	Diabetic Group	Non-diabetic group	Total
Polymicrobial	26	09	35
Monomicrobial	20	19	39
No growth	04	08	12

Overall Staphylococcus aureus was the single most common isolate, from 34 of 86 cases (40%). Non-sporing anaerobic gram negative bacilli were isolated

on 12 occasions, 10 of the cases were from diabetic group and only 2 from non-diabetic group.

Bacterial isolates

Organism	Diabetic Group	Non-diabetic Group
Staphylococcus aureus	18 (25%)	16 (43.2%)
E.coli	10 (13.8%)	02 (5.4%)
Proteus sp.	14 (19%)	04 (10.8%)
Pseudomonas aeruginosa	12 (16.6%)	06 (16.2%)
Citrobacter sp.	06 (8.3%)	03 (8.1%)
Klebsiella sp.	02 (2.7%)	01 (2.7%)
Total Gram Negative Isolates	44 (61 %)	16 (43.2%)
Corynebacterium sp.	00	03 (8.1%)
Non sporing anaerobes	10 (13.8%)	02 (5.4%)
Total Isolates	72	37

Comparatively diabetic and non-diabetic groups showed different trends. Staphylococcus aureus dominated the non-diabetic group as a single isolate from 16 cases (43.2%). In diabetic group Staphylococcus aureus was only 25% of the isolates. Gram negative bacilli were isolated on 44 occasions (61%) from diabetic group, while in non-diabetics it was limited to 43.2% only. Among the gram negative

bacilli, Proteus sp. was common (19%) in diabetics and in non-diabetics Pseudomonas sp. was detected in 16% cases. Corynebacterium sp. was isolated as a pure culture from 3 non-diabetic patients.

Repeated samples were received from 21 cases where the infection persisted. 19 of these were diabetic patients while from non-diabetics only in 2 cases sample was repeated. In all the repeated samples,

same organism was isolated. In 15 out of 19 diabetic patients where sample was repeated, second sample showed some additional organism along with the previous organism.

On antibiotic sensitivity testing 30% of the isolates from diabetic group were multidrug resistant. Only 2% of isolates from non-diabetic group were multidrug resistant. Most of the gram negative bacilli and gram positive cocci were sensitive to Amikacin. Clindamycin was also effective against gram positive cocci.

Discussion:

Management of non-healing ulcers always poses a great difficulty. Many cases, particularly from diabetic patients, need surgical intervention as well as proper targeted antibiotic therapy. For this, bacteriological study of these ulcers has been done by many workers in the past [5, 8, 10]. There are studies from India [1, 11] and also from abroad [4, 6, 12]. In the present study we have compared the bacterial etiological agents from patients with diabetes mellitus and patients without diabetes mellitus. Diabetic foot infections are classified on the basis of severity by Wagner [13].

Predominance of male patients has been reported in all the studies [8, 14], same is our finding. In diabetic group 34 out of 50 were males and in non-diabetics 28 out of 36 were males. The age range in diabetic group was 31-80years, mean being 55 years. Other reports have various age ranges with mean ages of 43 years [14] to 63 years [8]

In our study total of 109 aerobic bacteria were isolated while only 12 anaerobic organisms were grown. This is because we have excluded the patients with deeper wounds. We have included only Wagner grades 0, I and II patients and not any grades above that. 10 non-sporing anaerobes were from diabetic

group (13.8%) while 2 were from non-diabetics (5.4%). Pathare et al [8] have reported a total of 28.9% of anaerobes, but majority of these are from Wagner grades III and above. From Wagner grades I & II, it is only 13.4%, which is very similar to our findings. Another study by Anandi et al [14] reports absence of anaerobic organisms from diabetic foot ulcers of Wagner grades 0 and I.

In the present study, polymicrobial flora was detected from 52% of diabetic patients but in non-diabetics it was only in 25% cases. Polymicrobial flora is reported from diabetic foot ulcers by many authors [14, 15], while a few authors report predominance of monomicrobial infections [16, 17]. Amongst the bacterial isolates, in the diabetic group we found that gram negative bacilli (61%) outnumbered the gram positive cocci (25%). Many other reports support our findings [11, 16, 18]. Kavitha et al [16] have reported gram negative bacilli from 52.31% and Tiwari et al [11] have reported the same from 78% cases. There are a few authors who have reported otherwise. In those studies gram positive organisms are more frequently isolated [15, 19]. In our study, in the group of non-diabetic patients, *Staphylococcus aureus* is the single commonest organism (43%).

From three of the non-diabetic patients, *Corynebacterium* sp. was isolated as a pure culture. Though *Corynebacterium* sp. is a normal skin flora and is usually not considered pathogenic, there are reports which suggest that *Corynebacteria* in high number can cause skin infection and can be associated with delayed wound healing [5, 20]. The antibiotic sensitivity pattern showed that in diabetic group 30% of all the isolates (including gram positive and gram negative bacteria) showed multiple drug resistance. But in non-diabetic group only 2%

isolates were multidrug resistant. This is most probably due to more chronic nature of the ulcers in diabetic group and also due to the polymicrobial nature of the ulcers. 19 of the diabetic patients revisited the hospital as against 2 non-diabetic patients. Swabs were recollected in every visit. Every repeat sample showed more than one organism and more drug resistant organisms than the previous sample. This shows that more the chronicity of the ulcer, more the drug resistance in the organisms.

Conclusion:

From the present study we can conclude that the etiology of non-healing ulcers in diabetic patients is different than that of the non-diabetic patients. Gram negative bacilli are the main culprit in diabetic patients but in non-diabetic patients *Staphylococcus aureus* is the predominant causative organism. As the chronicity of the ulcer increases, the drug resistant bacterial population increases. Giving targeted treatment, after knowing the causative agent and its drug sensitivity pattern, is the best way to treat non-healing foot ulcers. This is better than empirical treatment.

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