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Original Research Article

A COMPARATIVE STUDY ON THE OUTCOME OF DIABETIC FOOT INFECTIONS TREATED ACCORDING TO DEEP TISSUE CULTURE AND SWAB CULTURE

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Abstract

Background: Diabetes-related foot infections occur in approximately 40% of diabetes-related foot ulcers and cause significant morbidity. The present study compares the efficacy of deep tissue culture and swab culture for treating diabetic foot infection (DFI). **Materials and Methods:** This study obtained two cultures from each patient after wound cleansing and debridement. The patients had not received systemic antibiotic therapy for at least four weeks before swabbing and deep tissue culture (DTC). Patients were followed up after debridement and antibiotic treatment, and data were recorded. Organisms were identified in different grades of ulcers and different wound culture settings. The healing response, including granulation tissue, wound discharge, surrounding skin, and pain intensity, was evaluated for both techniques at the end of a 25-day follow-up period. **Results:** In this study, 100 patients were enrolled, with 10 being excluded. The Tissue group had mostly grade 3 ulcers, while the Swab group had mostly grade 4 ulcers. Swabbing yielded a higher percentage of microorganisms compared to tissue sampling. After a 20-day follow-up, bacterial isolates decreased significantly in both groups. Staphylococcus aureus was common at enrollment, while coagulase-negative staphylococcus prevailed later. E. coli and proteus were commonly isolated Gram-negative organisms. The Tissue group had higher clinical and microbiological cure rates. Both groups showed good healing responses, but the Swab group had more persistent infections after 20 days. **Conclusion:** The study suggests that swabbing and biopsy of the ulcer base can be equally reliable for initial follow-up in limb-threatening diabetic foot infection if laboratory processing is adequate.

INTRODUCTION

Diabetic Foot infections (DFIs) are one of the leading causes of hospitalisation. It typically begins in neuropathic ulceration. Its prevention requires early detection and intervention.[1] It requires careful attention and coordinated management, preferably by a multidisciplinary foot-care team. The presence of infection is defined by more than or equals to 2 classic findings of inflammation or purulence. Conditions are then classified into Mild (superficial and limited in size and depth), Moderate (deeper or more extensive), and Severe (systemic signs or metabolic perturbations).[2]

Regardless of the type of diabetes classification, failure to achieve optimal glycemic control can cause damage to the body's small and large blood vessels and nerves. Damage to these vessels and nerves can affect all organs in the body.[3] These changes lead to a cascade of events resulting in changes to the foot itself. DFIs are polymicrobial, with staphylococci (aerobic gram-positive cocci), the MC causative organism. Many DFIs require surgical intervention, ranging from minor (debridement) to major (resection, amputation).[4-5] Non-healing chronic ulcers, despite daily dressing with local applications, do not heal. This problem is especially seen in diabetic, venous, and pressure

ulcers. Treating these wounds is a constant challenge for the surgeon.[6]

The peculiarity of a chronic wounds is that they refuse to heal. Wound debridement and dressings and improving the nutritional status are all important factors in wound healing. Various studies were done on dressings in the management of DFIs.[7] Despite all these, treating the microbes of a native wound is by far the most important and nidus in the management. Various debates are going on for the best method of specimen collection, whether swabbing or deep tissue culturing. Some studies proved deep tissue culture is the best method of identifying microbes. But still, in many peripheral, even tertiary centres, many clinicians follow the SWAB TECHNIQUE for microbes culturing.[8-9] This study was to compare the efficacy in identifying the organisms and the best method of specimen collection for culture study by comparing the culture of SWAB VS DEEP TISSUE.

MATERIALS AND METHODS

The hospital-based non-randomised controlled comparative study was conducted on 100 patients in GRH Madurai for four months. Patients of either sex ageing between 40 to 60 years having diabetic foot infections, were enrolled for the study. All 100 patients were divided into Group A (deep tissue culture) and Group B (swab culture). Institutional ethical committee approval and written consent were taken before the start of the study.

Inclusion Criteria

All patients who are willing to participate in the study with written consent. Patients aged 40- 60 years in both sexes presenting with diabetic ulcer foot of grade more than 1 (IDSA/IWGDF classification) were included.

Exclusion Criteria

Patients with Hb < 9gm, albumin < 3gm and creatinine >2, patients with an immunocompromised state other than diabetes, gangrene foot and non-palpable pulse in peripheries, and patients without consented to inclusion in the study were excluded.

Data Collection Methods

- Grading the patients according to IDSA/IWGDF classification, patients' grades of more than one are taken for this study.
- They were numbered from 1 to 100. Every alternate one was chosen. The odd one was subjected to the SWAB group, even number to the TISSUE group.

Methodology

Two cultures were simultaneously taken from each patient after the wound had been cleansed (using sterile saline and gauze) and debrided (removal of necrotic tissue, foreign material, calluses, and undermined wound edges) in the lack of systemic antibiotic therapy for at least four weeks before swabbing and deep tissue culture (DTC). No antimicrobial agent (e.g., alcohol or iodine) or antiseptic was introduced into the wound before specimen collection. Superficial swab cultures (SC) were taken using the Levine technique, rotating a wound swab over a 1 cm2 area of the wound for 5 seconds, using sufficient pressure to extract fluid from the inner part of the wound. DTC samples about 4 mm in diameter were taken from the junction of non-viable and viable tissue using forceps. All non-viable tissue was removed from the wounds, and the sinus tract or abscess extension was performed in the deep tissue debridement. Samples were inserted into a transport tube containing brain infusion broth suitable for aerobic and anaerobic microorganisms and delivered to the laboratory for immediate processing within 15 min after collection. Only one site was sampled from each patient. Culturing of aerobic and anaerobes species were inoculated onto blood agar, EMB (Eosin Metilen Blue) agar, Sabouraud agar and Wilkins-Chagren anaerobe agar at 35-37°C for 24-48 hours. The hemolysis reaction, catalase test, optochin, bacitracin and cotrimoxazole susceptibility testing was performed for Gram-positive bacteria, while oxidase tests were applied for gram-negative bacteria. Kirby Bauer Disc, diffusion sensitivity testing, was performed.

- The patient was given an empirical antibiotic initially then treated for infection according to swab C/S for the 'swab' group and deep tissue C/S for the 'deep tissue group.
- After thorough debridement and respective antibiotic coverage patient had to be followed up, and data was recorded.
- Now comparing the efficacy in managing DFIs by comparing the culture of swab technique and deep tissue biopsy method by analysing the outcome of their wound healing.

In this study of 90 patients, specimens were taken at admission (T1) and then started on Empirical antibiotics. From the 5th day onwards, culture-sensitive antibiotics (tissue group – tissue C/S, swab group – swab C/S) were given. Then the second specimen for the same patient was taken on the 11th day of admission (T11), and then on the 15th of admission, a revised culture-sensitive antibiotic was given. 3rd specimen was taken on 20th day (T20). The wound was thoroughly debrided for all patients, and the dressing was done. The wound was examined for healing response. For 90 patients, a total of 540 specimens (270 each for swabs and tissue) were taken. Patients with grade 4 ulcers mostly had underlying abscesses. These patients were treated under high care with proper wound debridement and higher antibiotics. The results obtained were statistically evaluated, and the main parameters which were analysed were;

- Organisms are identified in different grades of ulcers as well as in different settings of wound culture.

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- Healing response regarding granulation tissue, wound discharge, surrounding skin, and pain intensity (Lego pain assessment tool).

Statistical Analysis

The collected data was entered in Microsoft Excel (windows 10) and analysed using the statistical package for social sciences (SPSS-19). To find an association between two categorical variables Pearson chi-square test was used. The value of P <0.05 is considered statically significant.

RESULTS

Out of 100 patients, ten patients were excluded from the study because of the amputation, expired, or lost follow-up during the study. We lost two patients for follow-up; 3 patients passed due to sudden myocardial infarction, and 5 Underwent amputation who all had severe sepsis.

Table 1: Observation of demographic variable and Ulcer grades of patients

Parameters	Observation N (%)		P-value
	Group A (N=50)	Group B (N=50)	
Gender			
Male	89		-
Female	11		
Mean Age (years± SD)	50-55		0.654
Ulcer Grade			
2	11	15	0.405
3	20	14	
4	14	16	
3	20	14	
4	14	16	

Male predominance was reported in our study, and the mean age group of patients was 55 – 57 years. Most of the patients lie in the age group of >50. In the Tissue group, a maximum number of patients (20) were found in ulcer grade class 3, whereas in the group swab, most of the patients (16) were found in ulcer grade 4 [Table 1].

A total of 1107 microorganisms (an average of 12.3 per wound for three sittings of the specimen) were isolated from the swab and tissue specimens from 90 wounds. At the enrolment, Gram-positive bacteria were frequently isolated from the SWAB technique, whereas after the 20 days of follow-up, it was frequently isolated from the TISSUE biopsy technique. Gram-negative bacteria were frequently isolated from the SWAB technique and, after 20 days of follow-up, were frequently isolated from the TISSUE biopsy technique. The effect was statistically significant (p<0.05) [Table 2].

The prevalence of polymicrobial infection diagnosed by TISSUE culture increased from 28.9% for grade 2 wounds to 31.8% and 33% for grade 3 and grade 4 wounds, respectively, whereas for SWAB culture, it was 40.8%, 32.6% and 26.5% respectively. TISSUE shows a significant difference in the isolation of poly microbes compared to SWAB (P=0.047). The MC polymicrobial combination is E. Faecalis, Staph. Aureus, Pseudomonas.

Diptheroids were isolated at enrolment and after 20 days, follow-up in four cases by SWABBING and in no case by TISSUE biopsy. Citrobacter spp. was isolated at enrolment and after 20 days follow-up in 5 cases by TISSUE biopsy and in no case by SWABBING.

The overall numbers of bacterial isolates yielded from swabbing and tissue sampling were 55.8% and 44.2%, respectively. After 20 days, follow-up numbers of bacterial isolates yielded from swabbing and tissue sampling were 45.1% & 54.9%, respectively, statically significant (p<0.05) at the enrolment among Gram-positive microbes Staph. Aureus was the MC isolated species in TISSUE and SWAB, with percentages of 29.9% and 35.5%, respectively. After 20 days, follow up with Staph. Aureus was the MC isolated species, appearing in 35.8% of the tissue specimens, and coagulase-negative Staph was the MC one in 32.6% of the swab specimens. At the enrollment among Gram-negative microbes, E. coli and proteus were the MC isolated species in TISSUE and SWAB, with a percentage of 27% and 37.1%, respectively. Among the Gram-negative organisms, Proteus spp. was the most prevalent, isolated from 31.5% of the biopsied wounds, and pseudomonas were the MC one from swabbed wounds at about 34.1% after 20 days follow up [Figure 1].

Table 2: Observation of microorganisms and evaluation parameters of patients of both groups

Parameters	Observation N (%)		P-value
	Group A (Deep tissues Culture)	Group B (swab Culture)	
Microorganism			
Gram-positive bacteria			
<10 days	92	104	0.266
10-15 days	91	71	
15-25 days	92	89	
Gram-negative bacteria			

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<10 days	74	105	0.833
10-15 days	110	76	
15-25 days	124	88	
Evaluation of wound			
Edge comparison			
<10 days			
irregular	45	45	
10-15 days			
Punch	15	32	
Sloping	30	13	
16-25 days			-
Punch	8	18	
Sloping	6	15	
Floor comparison			
<10 days			
Slough	45	45	
10-15 days			-
Slough	15	32	
Granulation	30	13	
16-25 days			
Slough	8	18	
Granulation	6	15	
Surrounding skin comparison			
<10 days			
Erythema	45	45	
10-15 days			
Erythema	16	32	
Dec.	29	13	
16-25 days			-
Erythema	8	18	
Dec.	6	15	
Pain comparison			
<10 days			
High (+)	45	45	
10-15 days			
High (+)	16	32	
Decreased	24	7	
No pain	5	6	
16-25 days			
High (+)	8	18	-
Decreased	6	15	
Exudate comparison			
<10 days			
p	45	45	
10-15 days			
P/D	5	2	
Pus	16	30	-
s	20	11	
sd	4	2	
16-25 days			
Pus	10	18	
s	11	15	

After a 25-day follow-up, only 17 patients displayed both clinical and microbiological cures among Group A (TISSUE group) and only five patients among Group B (SWAB group) (P=0.041). In the present study, within 15 days of follow-up, 12 and 30 patients showed good healing responses among SWAB and TISSUE groups, respectively, and the effect was statistically significant (P=0.004) (Table 2). Even after 20 days of follow-up, 18 patients still have signs of infection in the SWAB group and only nine in the TISSUE group.

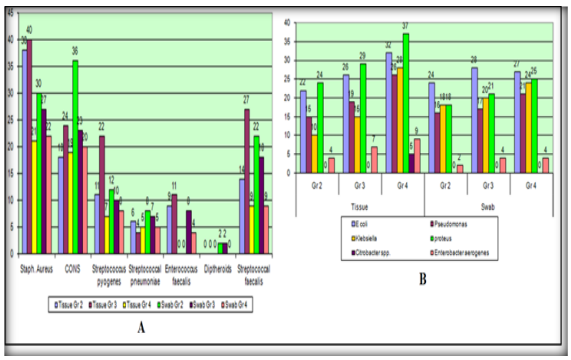

Figure 1: Observation of (A) Gram-positive and (B) Gram-negative bacteria in both group patients

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DISCUSSION

A reliable sampling technique is necessary to identify pathogens in infected diabetic foot wounds. A systematic review of the diagnosis of infections in diabetic foot ulcers has concluded that the available evidence is too weak to determine the optimal sampling technique. Most researchers consider tissue biopsy the best method for identifying pathogens in DFIs because deep biopsy is not prone to superficial contamination.[5-7]

Nelson et al. have conducted a large, prospective, multicenter trial to assess the concordance between the culture result for swabs and tissue specimens in patients with clinical DFIs.[10] Previous studies have ignored that the microbial species detected in wounds of varying depths and severities can significantly differ. Furthermore, the accuracy of swabbing has not been assessed to the PEDIS infection grade. Thus, we reappraised the concordance between swabs and tissue culturing according to the PEDIS infection grade of diabetic foot wounds. Pellizzer et al. found the mean number of isolates per patient as 2.34 by swabbing and 2.07 by tissue biopsy sampling.[11] Kessler et al. found the mean number of microorganisms isolated by needle puncture significantly lower than that obtained by superficial swabbing: 1.09 vs. 2.04 ($P<0.02$). However, they also observed that the swab specimen identified 13 microorganisms (62%) isolated from the needle puncture culture.[12]

In this study, there was a significant difference in several isolates identified by TISSUE and SWAB. The SWAB isolated more microbes at the enrolment than TISSUE, but TISSUE was better for chronic infections. But the number of isolates per patient by TISSUE (1.84 to 2.4) was increased in 25 days follow-up compared to SWAB (2.32 to 1.3) but was statistically insignificant.

Our results are not compatible with Bill et al. study reports.[13] They reported that swabs do not accurately identify bacterial pathogens in diabetic foot wounds. However, these studies were restricted to patients who underwent amputation. Therefore, the poor performance of swabs in these studies might have been due to the excessive growth of colonisers at the site of the wound after the foot or limb had lost its viability. In contrast, our protocol excluded specimens from infectious gangrene and amputations.

As with Pellizzer et al., this study denotes Gram-negative microbes have been better isolated by TISSUE biopsy than SWAB in chronic infection patients.[11] As the chronicity (GRADE 2 - 23.1 to 38.5%, GRADE 3 – 75% to 97.5%) and Grading of ulcer increases, SWAB lacks to isolate microbes as the TISSUE can. Out of 45 patients, 17 patients have both clinical& microbiological cures in the TISSUE group compared to only 5 in the SWAB group. This shows significant improvement in managing DFIs by treating the patients with TISSUE C/S antibiotic and in the healing response.

Few studies have prospectively compared superficial swabbing with deep tissue culture in the microbiological monitoring of severe diabetic foot.[9,12] Polymicrobism and anaerobic infection appear to be the features that most closely correlate with the severity of the clinical setting. Therefore, whatever sampling method is used, it should be sensitive enough to detect the range of potential pathogens and prevent the loss of obligate anaerobes. When infection is unresolved after standard treatment, the microbiological features of severe polymicrobial ulcers tend to resemble those observed in typically monomicrobial infections of superficial ulcers, and Gram-positive species, particularly staphylococci, are frequently isolated. It is unclear whether the higher prevalence of Gram-positive species detected in properly treated long-standing ulcers represents a marker of either colonisation or true infection. Indeed, the presence of facultative pathogens and S. aureus has been frequently observed to be associated with delayed wound healing, and a study by Bowler and Davies et al. has suggested that the role of synergistic microbial interactions in the pathogenesis of chronic wound infection may be of greater clinical importance than the isolated involvement of any specific potential pathogen.[14]

Limitations of the study:

The most important limitation of the present study is the technical issues in identifying the anaerobic culture. We couldn't determine that because of the patient's financial status and lack of resources during the project work. But we covered all the patients with available anaerobes covering antibiotics.

CONCLUSION

In conclusion, our experience suggests that swabbing and biopsy of the ulcer base may be equally reliable for the initial follow-up of empirical therapy in limb-threatening diabetic foot infection, provided that laboratory processing is adequate. In contrast, the microbiology of foot ulcers still active after two weeks of appropriate treatment appears better assessed by deep tissue culturing. Swab cultures may be reliable for guiding the antibiotic treatment of diabetic patients with grade 2 foot wounds. However, it is necessary to perform deep tissue biopsy for wounds of grade ≥ 3. In such cases, swab culturing is associated with a high risk of missing pathogens, especially Gram-negative bacteria.

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