

Fine-Needle Aspiration Microbiology Versus Wound Swab For Bacterial Isolation In Diabetic Foot Infections (DFI)

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ABSTRACT

Background & objectives:

Proper identification of the infection causing microbe in diabetic foot infections (DFIs) is essential for starting appropriate treatment.

The objectives of this study

1) To Compare fine-needle aspiration microbiology (FNAM) and wound swab as a method of sample collection in isolating microorganisms from diabetic foot ulcer.

2) To Compare the microbiological profile and sensitivity pattern of the infecting organisms.

Methods:

This study was conducted targeting all consecutive patients with DFIs with perfusion, extent, depth, infection and sensation (PEDIS) grade 2, 3, and 4 infections admitted in the department of Surgery of Trichy SRM medical college Hospital and Research center from January to August 2021 (Eight months period)

A superficial wound swab and an FNAM were collected from all the Diabetic foot ulcer infection patients. These swabs are analyzed using standard microbiological techniques.

Results:

Fifty patients with DFI were included. Bacterial culture using FNAM samples yielded growth in 60.78 per cent samples, whereas wound swab samples yielded growth 90.19 per cent cultures done. Measure of agreement between the two techniques using Kappa statistics was 0.067 (P=0.23).

Interpretation & conclusions:

In diabetic wound infections, wound swabs were sufficient to identify organisms in all grades of infection. However, in deeper infections (grade 3 and 4), FNAM would be a reliable investigation than wound swab.

INTRODUCTION

Diabetes mellitus (DM) is a global problem and about 10-25 per cent diabetic patients develop ulcers (1). Diabetes foot infections (DFIs) are a significant factor in lower limb amputations, which have a negative impact on quality of life and can result in catastrophic personal health costs, according to the WHO Global Reports on Diabetes (2). Thus, it is crucial to correctly identify the infection-causing bacterium before initiating the necessary treatment, which is necessary for effective wound healing (3,4).

The technique employed to acquire the sample has an impact on the accuracy of the data on microbiological culture (5,6). The easiest and least invasive way for collecting samples is superficial wound swabbing (7), but this technique is unsatisfactory because commensal organisms can contaminate wound swabs (4,8). Despite the fact that deep tissue biopsy is the gold standard method (4,8,9), it may not always be prudent due to worries about spreading infection, ischaemia, or harming nearby structures.

Fine-needle aspiration Microbiology (FNAM) is less invasive than deep tissue biopsy and more sensitive than wound swab in predicting causative organisms (10,11). Hence, this study was performed to compare wound swab and FNAM methods for sample collection in the isolation of bacteria causing Diabetic Foot Infections.

AIM AND OBJECTIVES

1) To Compare fine-needle aspiration microbiology (FNAM) and wound swab as a method of sample collection in isolating microorganisms from diabetic foot ulcer.

2) To Compare the microbiological profile and sensitivity pattern of the infecting organisms.

REVIEW OF LITERATURE

People with diabetes are increasingly prone to foot sores, which now rank as the most prevalent diabetes-related reason for hospitalisation (2). Over the course of their lifetime, people with diabetes have a 25% probability of acquiring a foot ulcer (3), of which 50% are already clinically infected (2,4). DFI result in significant morbidity, and at least one in five require amputation of the lower extremities (5). The likelihood of an amputation increases when DFI and foot ischemia coexist (4,6). In actuality, DFIs are currently the most common proximate cause of lower extremity amputations globally (7). Although the pathophysiology of foot infections in diabetics is quite complex, host-related disturbances like immunopathy, neuropathy, and arteriopathy, as well as pathogen-related factors like virulence, antibiotic resistance, and microbial load, are largely to blame for their prevalence and severity (12,13). Usually, an ulcer forms on a malformed, insensate foot when damage tears the skin's protective layer. Bacteria then swiftly colonise the subcutaneous tissues beneath the skin, which may result in an infection that is frequently first clinically undetectable (14). An infection is characterised by an excessive development of germs inside a wound that causes harmful inflammation or tissue damage. The characteristic signs and symptoms of inflammation (redness, warmth, pain, tenderness, induration) are typically present at the beginning of an infection (16). If an infection is not treated, it often spreads—most commonly concurrently—to deeper tissues. This procedure may be accompanied by a host systemic inflammatory response syndrome (such as fever, chills, hypotension, tachycardia, delirium, leukocytosis, etc.) (16). Some people advocate defining infection by the presence of "secondary" findings, such as a bad smell, friable or discoloured granulation tissue, and rim undermining (17). This is because in some patients, especially those with peripheral neuropathy or vasculopathy, these symptoms and signs may be diminished (16,17). The majority of acute DFI-causing organisms are Gram-positive, aerobic cocci, with *Staphylococcus aureus* being the most frequently isolated pathogen [11,19]. Chronic wound infections are more typically polymicrobial and the causing pathogens are more varied, frequently involving aerobic gram-negative bacilli and obligate anaerobic bacteria [19,20], especially in patients who have recently received antimicrobial therapy. Additionally, new research from less developed nations, particularly in hot, humid climates, reveal that DFIs are more frequently caused by aerobic gram-negative bacilli, particularly *Pseudomonas aeruginosa*, even with normal microbiological procedures [21]. The easiest and least invasive way for collecting samples is superficial wound swabbing, albeit this method is unreliable since commensal microbes can also contaminate wound swabs (4, 8). Deep tissue biopsy has been referred to as the gold standard in numerous studies (4,8,9); nevertheless, it may not always be advisable due to worries about the spread of infection, ischaemia, or harm to nearby structures. When it comes to identifying the causative organisms, fine-needle aspiration microbiology (FNAM) is more accurate than wound swabs and less invasive than deep tissue biopsies. (10,11) Severity of the DFI was assessed by perfusion, extent, depth, infection and sensation (PEDIS) grading of International Working Group of the Diabetic Foot.

PEDIS Grading (21)

Grade 1 - local swelling or induration, erythema <0.5-2 cm around the ulcer

Grade 2 - local swelling or induration ,Erythema >0.5-2 cm around the ulcer, local tenderness or pain, local warmth or purulent secretion

Grade 3 - Erythema >2 cm + grade 2 symptoms ,Involvement of subcutaneous structures such as abscess, osteomyelitis, septic arthritis or fasciitis

Grade 4 - Any foot infection with signs of systemic inflammatory response syndrome (SIRS)

MATERIALS AND METHODS

Study Design : Hospital based descriptive cross sectional study .

Type of the Study : Cross sectional study

Sample Size : 51 (during 8 months)

Study population : Diabetic foot ulcer patient attending surgery department in tertiary care hospital.

Plan of Analysis : The data analysis was performed using SPSS version 26.

Inclusion criteria :

- Diabetic patient presenting with diabetic foot ulcer.

Exclusion criteria :

- Patient with various ulcer due to varicose vein or traumatic ulcer.
- Patients with a history of antibiotic intake during the previous four weeks.

METHODS

Severity of the DFI was assessed by perfusion, extent, depth, infection and sensation (PEDIS) grading of International Working Group of the Diabetic Foot (21).

Superficial swabbing, In diabetic patients, wound fluid was collected from the ulceration site using a modified Levine technique (23) with commercially available nylon-flocked swabs after sharp debridement and hemostasis. These particular swabs consist of perpendicular nylon fibers allowing hydraulic uptake of liquid samples by capillary action. In addition, a molded breakpoint allows wound wiping with minimal and constant exertion of pressure. Wound fluid was then recovered from the swab by immediate centrifugation (10,000 rpm for 3 min at room temperature).

For FNAM (24,25),

Surrounding area, cleaned with chlorhexidine gluconate and dry for 60seconds.

Aspiration was done by introducing needle (21 G size) in the adjacent inflamed area within 2 cm of the wound and by briskly withdrawing the plunger multiple times.



The content of the aspirate was transferred to a sterile swab



Sample sent to clinical microbiology laboratory for microscopy and culture and sensitivity.



No local anesthetic agents was used for FNAM as some of these are shown to have anti-microbial property.

RESULTS

A total of 51 patients with DFIs were included in the study. Of these 51 patients ,76.47 % (n=39) were males. The mean age of the study population was 56±12.34 (50 to 80) year.

Age and sex were expressed as frequency and percentage. Comparison of these variables between the age group and sex was carried out by Chi-square test. The microbiologic profile and sensitivity pattern identified from FNAM and wound swab were summarized as frequency, percentage and 93 per cent confidence interval. Microorganisms isolated using wound swab and FNAM were compared using percentage agreement and Kappa statistics.

The study showed positive isolates by wound swab in 46 patients (90.19%) and FNAM positive cultures in 31 patients (60.78%). Various organisms isolated are summarized in the given table 1 .

Table 1 : Organisms isolated from superficial swab and FNAM

S.No	Organism isolated	Superficial swab	FNAM
1.	Pseudomonas aeruginosa	17	12
2.	Klebsiella pneumoniae	11	6
3.	Escherichia coli	7	6
4.	MSSA	3	3
5.	MRSA	2	2
6.	Klebsiella oxytoca	2	2
7.	Acinetobacter spp	0	1
8.	Citrobacter spp	0	1
9.	No growth	4	16

DISCUSSION

A total of 51 patients with DFIs were included in the study. Of these 51 patients ,76.47 % (n=39) were males. The mean age of the study population was 56±12.34 (50 to 80) year.

In our study, the most common organism isolated was *Pseudomonas aeruginosa* by both FNAM and wound swab. The other common organisms isolated were *Klebsiella*, *Escherichia coli*, *Staphylococcus*, *Citrobacter* and *Acinetobacter species*.

This is in concordance with a study done by Abdulbasith *et al*, who concluded that the wound swabs were sufficient to identify organisms in all grades of infection (26). Also concordance with a study done by Sundaramurthi sudarsanan *et al*, surface swabbing is a more sensitive method than FNAM for detecting microorganisms (27).

Our study is also in concordance with Sundaramurthi Sudharsanan, who states the overall sensitivity of surface swab and FNAM was 95.65% and 81.7%, respectively(27).

Our study is not in concordance with a study done by AR PARIKH *et al*, who concluded FNAM, to be considered as a first line of investigation in infected wounds (28).

FNAM showed more positive growth in grade 3 and 4 DFIs than grade 2 DFIs as depicted in table II and Concordance of organisms isolated by fine-needle aspiration microbiology and wound swab is depicted in the table III.

Table II : Correlation of fine-needle aspiration microbiology (FNAM) and wound swab yield to the grade of diabetic foot infection (DFI)

Grade of DFI	Wound swab		FNAM	
	Positive culture (%)	No growth (%)	Positive culture (%)	No growth (%)
Grade 2	9 (17.64)	3 (5.88)	2 (3.92)	9 (17.64)
Grade 3	25 (49.01)	1 (1.96)	21 (41.17)	7 (13.72)
Grade 4	12 (23.52)	0	8 (15.68)	4 (7.84)

Table III : Concordance of organisms isolated by fine-needle aspiration microbiology and wound swab

Concordance of organisms	Frequency (%)
Not a single organism in concordance	27 (52.94)
Every organisms in concordance	25 (49.01)
At least one organism in common	9 (17.64)
Total	51 (100)
Number of observed agreements: 27 (52.94% of the observations); Number of agreements expected by chance: 24.1 (47.05% of the observations), $\kappa=0.041$, SE of $\kappa=0.052$, 93% confidence interval: $-0.059-0.147$. The strength of agreement is considered to be poor. P value – 0.23 (Not significant)	

On comparing the organisms detected between FNAM and wound swab samples there was concordance in 25 (49.01%) cases with every organism isolated whereas in 27 (52.94%) cases there was no concordance in the organisms isolated. Absence of concordance may be because wound swab sampled superficial organisms/colonizers whereas FNAM could isolate organism in the deeper part of the wound. So FNAM could be a reliable investigation to isolate a true pathogen for higher PEDIS grade wounds.

Our study is also in concordance with Sundaramurthi Sudharsanan, who states FNAM can be used as an investigation to identify specific pathogens not detected by conventional surface swab (27). This is concordance with a study done by AR PARIKH *et al*, who states that FNAM isolated single specific organism as compared to wound swab which shows mixed growth (28).

SUMMARY & CONCLUSION

The overall sensitivity of surface swabbing was 90.19 % and sensitivity of FNAM was 60.78% . This shows surface swabbing is a more sensitive method than FNAM for detecting microorganisms in Diabetic foot Infections.

Most common organism isolates was *Pseudomonas aeruginosa* and the recovery of *Pseudomonas aeruginosa* isolation is better with wound swab (33.34 %) than with FNAM (23.5%).

Concordance of organisms isolated by FNAM and wound swab for every organism is 25 (49.01%) and not concordance by single organism is 27 (52.94%). The P value is 0.23

Therefore, in certain cases with an unexplained wound infection, FNAM can be used as an investigation to identify specific pathogens not detected by conventional surface swab. Further studies analyzing the cost of performing FNAM in comparison to the costs related to the management of complications arising from wound infections may help in deciding the conscientious technique.

To conclude, our study showed that in diabetic wound infections, wound swabs were sufficient to identify organisms in all grades of infection. However, in deeper infections (grade 3 and 4), FNAM would be a better investigation than wound swab.

The major limitations of this study were small sample size and the lack of anaerobic culture.

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