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Study of fine-needle aspiration microbiology versus wound swab for bacterial isolation in diabetic foot infections

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***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 were to compare fine-needle aspiration microbiology (FNAM) with wound swab as methods of sample collection in isolating microorganisms causing DFIs and also 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 a tertiary care hospital in south India during July to August 2017. A superficial wound swab and an FNAM were collected from all the patients. These swabs are analyzed using standard microbiological techniques.**

***Results*: Eighty patients with DFI were included. Bacterial culture using FNAM samples yielded growth in 58.75 per cent samples, whereas wound swab samples yielded growth in 93.8 per cent cultures done. Measure of agreement between the two techniques using Kappa statistics was 0.069 (*P*=0.28).**

***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.**

Key words Culture - diabetic foot infections - fine-needle aspiration microbiology - microorganism - wound swab

Diabetes mellitus (DM) is a global problem and about 10-25 per cent diabetic patients develop ulcers[1]. According to the WHO Global Reports on Diabetes[2], "diabetic foot infections (DFIs) are an important cause of lower limb amputation which have significant impact on quality of life and can also incur catastrophic personal health expenditures". Proper identification of the infection causing microbe is thus essential for starting appropriate treatment, which is required for proper wound healing[3,4]. The method used for the collection of sample influences the quality of data on microbiological culture[5,6]. Most commonly used method for sample collection is superficial wound swab for its ease and noninvasiveness[7], although unreliable, since wound swabs may also be contaminated by commensal organisms[4,8]. Many

studies have suggested deep tissue biopsy as the gold standard[4,8,9] but may not be always advisable due to concerns of spreading infection, ischaemia, or damaging adjacent 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 DFIs.

Material & Methods

The present study was conducted among consecutive DFI patients admitted in the department of Surgery, Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), a tertiary care centre in Puducherry, India, from July 1 to August 31, 2017. The study protocol was approved by the Institutional Ethics Committee and written informed consent was obtained from all participants.

Severity of the DFI was assessed by perfusion, extent, depth, infection and sensation (PEDIS) grading of International Working Group of the Diabetic Foot[12]. Patients with any two of the following signs such as local swelling or induration, erythema >0.5-2 cm around the ulcer, local tenderness or pain, local warmth or purulent secretion were graded as PEDIS grade 2. Patients with erythema >2 cm along with any one of the signs of grade 2 infections or infection involving structures deeper than skin and subcutaneous structures such as abscess, osteomyelitis, septic arthritis or fasciitis were graded as PEDIS grade 3. Any foot infection with signs of systemic inflammatory response syndrome (SIRS) was graded PEDIS 4.

Patients with a history of antibiotic intake during the previous four weeks, those with DFIs associated with dry gangrene and patients not willing to give consent were excluded from the study. At first, superficial wound swab was taken using Levine technique[13]. For FNAM, the surrounding non-ulcerated inflamed area within 2 cm of the wound was first cleaned with chlorhexidine gluconate and allowed to dry for 60 seconds. Fluid was aspirated from the suspected area using a 5 ml syringe and a 21G needle. Aspiration was done by introducing needle 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 wound swab. These swabs were sent to clinical microbiology laboratory for microscopy and culture and sensitivity using standard microbiological techniques. No local anesthetic agents was used for FNAM as some of these are shown to have anti-microbial property[14,15].

Statistical analysis: The data analysis was performed using Statistical Package for the Social Sciences version 20 (IBM SPSS, Chicago, IL, USA). 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 95 per cent confidence interval. Microorganisms isolated using wound swab and FNAM were compared using percentage agreement and Kappa statistics.

Table I. Isolates identified by fine-needle aspiration microbiology (FNAM) and wound swab samples

Organism isolated	FNAM	Wound swab
Gram-negative organism		
Acinetobacter baumannii	9	18
A. lwoffii	1	1
Citrobacter freundii	1	1
C. koseri	-	1
Enterobacter species	5	5
Escherichia coli	13	21
Klebsiella pneumoniae	8	13
Morganella morganii	1	1
Non-fermenting Gram-negative bacilli	-	1
Proteus mirabilis	2	8
P. penneri	-	1
P. vulgaris	1	-
Providencia rettgeri	-	1
Pseudomonas aeruginosa	5	11
Pseudomonas species	6	11
Gram-positive organism		
Beta-haemolytic streptococci group D	1	1
Beta-haemolytic streptococci group G	1	1
Beta-haemolytic streptococci group F	1	1
Coagulase-negative *Staphylococcus aureus*	-	1
Enterococcus faecalis	1	2
S. aureus	8	12
Streptococcus species	2	2

Results & Discussion

A total of 80 patients with DFIs were included in the study. Of these 80, 72.5 per cent (n=58) were males. The mean age of the study population was 56±12.34 (27 to 80) yr. The study showed positive isolates by wound swab in 75 patients (93.8%) and FNAM-positive cultures in 47 patients (58.75%). Various organisms isolated are summarized in Table I. This was in concordance with a study done by Gjødsbøl *et al*[16], who concluded that it was sufficient to use swab specimens to identify the bacterial species present in the chronic wounds. Demetriou *et al*[17] showed that swab cultures were highly sensitive but less specific and had good negative predictive value in diabetic patients.

In our study, the most common organism isolated was *Escherichia coli* by both FNAM and wound swab. The other common organisms isolated were *Acinetobacter*, *Klebsiella*, *Pseudomonas*, *Enterobacter* and *Staphylococcus*. FNAM showed more positive growth in grade 3 and 4 DFIs than grade 2 DFIs as depicted in Table II. However, this did not attain significance owing to the small sample size of the study. The diagnostic accuracy of FNAM could not be established due to lack of gold standard (tissue culture) in our study. On comparing the organisms detected between FNAM and wound swab samples there was concordance in 32 (40%) cases with every organism isolated whereas in 37 (46.25%) cases there was no concordance in the organisms isolated (Table III). 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.

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

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

Grade of DFI	FNAM (n=80)		Wound swab (n=80)	
	Positive culture (%)	No growth/ NSFG (%)	Positive culture (%)	No growth/ NSFG (%)
Grade 2	3 (3.75)	15 (18.75)	14 (17.50)	4 (5.00)
Grade 3	27 (33.75)	11 (13.75)	37 (46.25)	1 (1.25)
Grade 4	17 (21.25)	7 (8.70)	23 (30)	0

NSFG, normal skin flora grown

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

Concordance of organisms	Frequency (%)
Not a single organism in concordance	37 (46.25)
Every organisms in concordance	32 (40.00)
At least one organism in common	11 (13.75)
Total	80 (100)

Number of observed agreements: 37 (46.25% of the observations); Number of agreements expected by chance: 35.1 (43.81% of the observations), κ=0.043, SE of κ=0.053, 95% confidence interval: −0.061-0.148. The strength of agreement is considered to be poor

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***Conflicts of Interest*:** None.

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