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Comparative study of tissue culture and sensitivity versus swab culture and sensitivity of microorganisms in the healing of diabetic foot ulcers

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Abstract

This prospective observational study, conducted at the Department of General Surgery, SRM Medical College and Hospital, aimed to assess the effectiveness of tissue culture and sensitivity compared to swab culture and sensitivity in the healing of diabetic foot ulcers through antibiotic sensitivity of microorganisms. Between May 2016 and August 2017, 160 subjects with diabetic foot ulcers were randomly assigned treatment based on either swab or tissue culture findings. Patients were followed at 15-day intervals for up to 60 days. Results showed positive swab cultures in 76.88% and positive tissue cultures in 92.50% of the study population. The most prevalent organism in swab cultures was Proteus (14.38%), while Pseudomonas (16.88%) dominated in tissue cultures. The cumulative proportion of subjects developing granulation tissue was faster in the tissue culture group, reaching 57.50% at 15 to 30 days and 99% at 31 to 45 days. The swab culture group exhibited proportions of 48.80%, 75%, and 93.80% at the same intervals. In conclusion, diabetic foot ulcer treatment based on tissue culture showed slightly faster healing rates compared to swab culture. However, both groups achieved good ulcer healing within the 60-day follow-up period. These findings emphasize the importance of choosing an appropriate culture method for effective management of diabetic foot ulcers.

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INTRODUCTION

Diabetes mellitus is described as a group of metabolic disorders characterized by increased blood glucose concentration. It is fast emerging as one of the major public health problems, with a significant burden of morbidity and mortality across the globe. Type 2 Diabetes Mellitus makes up about 85-90% of all cases [1][2][3][4]. Diabetic foot ulcer is a major complication of diabetes mellitus. Nowadays, treating clinicians rely more on microbiological data than clinical data to diagnose infection. The isolation of the causative organism is the primary step in the management of infected diabetic foot ulcers. Microbiological

samples from infected diabetic foot ulcers are obtained by either tissue biopsy or by taking wound swab [5][6]. Superficial wound swabbing has been applied more widely in day-to-day clinical practice because of its non-invasive nature and ease of performance [7][8][9].

A systematic review of the diagnosis of infections in diabetic foot ulcers concluded that the evidence was too weak to determine the optimal sampling technique [5]. The evaluation of agreement between culture results obtained from swab and those obtained from tissue specimens among infected diabetic foot ulcers to identify the microbiological profile is a valuable research question that needs to be answered. The objective of our study is to compare tissue culture and sensitivity versus swab culture and sensitivity of microorganisms in the healing of diabetic foot ulcers [10][11][12][13][14].

MATERIALS & METHODS

Study Design: The current study was a prospective observational study.

Study Setting: The study was conducted in the Department of SRM Medical College, Hospital, and Research Centre.

Study Population: The study population included patients presenting with diabetic foot, who underwent diagnostic evaluation either by tissue culture or swab culture.

Inclusion Criteria:

1. Age above 18 years.
2. Willingness to participate in the study.
3. Patients admitted to the Department of General Surgery with Diabetic Foot Ulcers.

Exclusion Criteria:

1. Patients administered antibiotics within 72 hours will be excluded from the study.
2. Transportation of samples taking more than 6 hours will be discarded and excluded from the study.

Study Period: The data collection for the study was done between May 2016 to August 2017.

Sample Size: The sample size was calculated assuming the proportion of culture-positive infections as 89% as per the study by Huang, Y., et al. The other parameters considered for sample size calculation were 5% precision and 95% confidence level. The following formula was used for sample size calculation:

$$n = \frac{Z^2\, P(1-P)}{d^2}$$

Where:

- n = Sample size
- Z = Z statistic for a level of confidence = 1.96
- P = Expected prevalence of proportion (If the expected prevalence is 20%, then P=0.89), and
- d = Precision (If the precision is 5%, then d=0.05).

As per the above-mentioned calculation, the required sample size was 151 subjects. To account for loss to follow up of 5%, it was decided to include another 8 subjects. Hence, the total required sample size was 159. The final analysis of the study included 160 subjects.

Sampling Method: All the study subjects were included in the study by convenient sampling until the sample size was reached. Out of the included study subjects, half of the subjects were treated based on swab culture results as per the institutional protocol, and the remaining people received treatment based on tissue culture, which was done randomly.

Study Procedure: For all patients in the study group, tissue culture and swab culture were done. Tissue biopsy of size 1x1cm was taken from the base of the ulcer and transported in a sterile container within 6 hours to the laboratory.

Period of Follow-up: Patients were followed at 15 days' intervals until the ulcer granulated.

Methods of Data Collection: The data were collected using a structured study proforma designed exclusively for the purpose of the study.

Ethical Considerations: Clearance was obtained from the ethical committee of SRM Medical College Hospital and Research Centre – Tamil Nadu for the study. Written and informed consent were sought from the patients and their attendants. They were given the option of quitting the study if so desired by them. No element of compulsion was exerted. All data were kept confidential.

Statistical Methods: Timing of appearance of granulation tissue was considered the primary outcome variable. Type of organism isolated in culture, the need for repeat cultures, and reduction in the size of the ulcer, etc., were considered as other outcome variables [9][13][15].

Sample collection method for culture (Swab Vs Tissue culture) was considered the primary explanatory variable.

Age, gender, duration of diabetes, baseline glycemic control parameters like HbA1C, and baseline size of the ulcer, etc., were considered as other relevant parameters.

Descriptive Analysis: Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency and proportion for categorical variables. Data were also represented using appropriate diagrams like bar diagrams, pie diagrams, and box plots. Both study groups were compared with respect to all potential confounding variables using Mean ± SD for quantitative variables using independent sample t-test. Categorical variables were compared using frequency and percentage, and the chi-square test was used to assess statistical significance.

The proportion of people developing granulation tissue at different follow-up periods was assessed and compared. The cumulative proportion of people developing granulation tissue was compared between the two groups using a compared trend diagram.

A p-value < 0.05 was considered statistically significant. IBM SPSS version 22 was used for statistical analysis.

RESULTS

A total of 160 subjects were included in the study. Out of these, 80 subjects were treated based on the results of tissue culture, and 80 people were treated based on the results of swab culture.

- The mean age of the individuals in the swab culture group was 55.98 ± 11.71 years, while in the tissue culture group, it was 54.31 ± 12.11 years. The difference in age between the two groups was statistically not significant (P value 0.379).
- In the swab culture group, 48 (60%) were male, and 32 (40%) were female. In the tissue culture group, there were 50 (62.5%) males and 30 (37.5%) females. The gender distribution difference between the two groups was statistically not significant (P value 0.746).
- The mean HbA1c (gm %) in the swab culture group was 8.89 ± 0.91, and in the tissue culture group, it was 8.64 ± 1.07. The difference in HbA1c (gm %) between the two groups was statistically not significant (P value 0.114).
- Among those in the swab culture group, 41 (51.25%) had hypertension, and 39 (48.75%) had no hypertension. In the tissue culture group, there were 46 (57.5%) with hypertension and 34 (42.5%) without hypertension. The difference in hypertension proportion between the two groups was statistically not significant (P value 0.427).
- The mean baseline area of the ulcer in the swab culture group was 39.38 ± 23.86 cm^2, and in the tissue culture group, it was 41.36 ± 37.48 cm^2. The difference in the baseline area of the ulcer between the two groups was statistically not significant (P value 0.690).
- Among the study population, 123 (76.88%) had a positive swab culture, and 37 (23.13%) had a negative swab culture.

Table 1 Descriptive analysis of different organisms isolated in swab culture (N=160)

Organism isolated in swab culture	Frequency	Percentage
Proteus	23	14.38%
Pseudomonas	19	11.88%
E.coli	13	8.13%
Acinetobacter	13	8.13%
Staphylococcus aureus	11	6.88%
Enterococcus	11	6.88%
Klebsiella Pneumoniae	10	6.25%
Streptococcus	9	5.63%
MR-CONS	6	3.75%
MRSA	5	3.13%
Commensals	3	1.88%
No organism isolated	37	23.13%

The most commonly isolated organism was Proteus, accounting for 23 (14.38%) of the study population. Other common isolates in swab culture included Pseudomonas in 19 (11.88%) cases, E. coli in 13 (8.13%), and Acinetobacter in another 13 (8.13%) individuals (**Table 1**).

Among the study population, 148 (92.50%) tested positive in tissue culture, while 12 (7.50%) tested negative.

Table 2 Descriptive analysis of different organisms isolated in tissue culture (N=160)

Organisms isolated in Tissue culture	Frequency	Percentage
Pseudomonas	27	16.88%
Proteus	23	14.38%
Klebsiella Pneumoniae	21	13.13%
Staphylococcus aureus	17	10.63%
E.coli	17	10.63%
Enterococcus	13	8.13%
Acinetobacter	12	7.50%
MRSA	8	5.00%
Streptococcus	6	3.75%
MR-CONS	4	2.50%
No organism isolated	12	7.50%

Among the study population, Pseudomonas was the most commonly isolated organism in tissue culture, occurring in 27 (16.88%) individuals. Other common organisms isolated were Proteus, Klebsiella Pneumoniae, Staphylococcus aureus, and E. coli in 23 (14.38%), 21 (13.13%), 17 (10.63%), and 17 (10.63%) people, respectively (**Table 2**).

Table 3 Profile of Mixed organisms isolated in swab culture (N=160)

Swab culture mixed organism	Frequency	Percentage
No mixed organism	123	76.88%
Staphylococcus aureus+ E.coli	10	6.25%
Klebsiella + staphylococcus	16	10%
Proteus +E.coli	6	3.75%
Pseudomonas +E.coli	5	3.13%

Among the study population, 123 (76.88%) did not have mixed organisms in culture. In 16 (10%) of the subjects, both Klebsiella and Staphylococcus were isolated. Staphylococcus aureus + E. coli were found in 10 (6.25%) individuals. Proteus + E. coli was isolated in 6 (3.75%), and Pseudomonas + E. coli was isolated in 5 (3.13%) subjects, respectively (**Table 3**).

Table 4 Profile of mixed organisms isolated in Tissue culture (N=160)

Tissue culture mixed organism	Frequency	Percentage
No mixed organism	149	93.13%
Staphylococcus aureus+ E.coli	5	3.13%
Staphylococcus +Klebsiella	2	1.25%
Pseudomonas +E.coli	2	1.25%
Klebsiella +staphylococcus	2	1.25%

In tissue culture, 149 (95.13%) of the study population did not have mixed organisms. The number of people with Staphylococcus aureus + E. coli, Staphylococcus + Klebsiella, Pseudomonas + E. coli, and Klebsiella + Staphylococcus isolates was 5 (3.13%), 2 (1.25%), 2 (1.25%), and 2 (1.25%), respectively (**Table 4** & **Figure 1**).

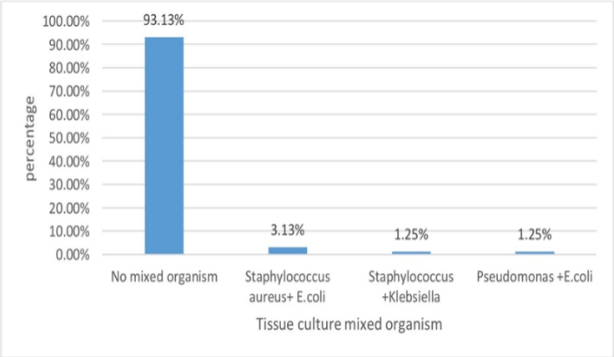

Figure 1 Bar chart of Tissue culture mixed organisms distribution in study population (N=160)

Table 5 Comparison of timing of granulation tissue in both the treatment groups (N=160)

Timing of granulation tissue	Treatment group	
	Swab (N=80)	Tissue culture (N=80)
15 to 30 days	39 (48.7%)	46 (57.5%)
31 to 45 days	21 (26.25%)	33 (41.25%)
45 to 60 days	15 (18.75%)	0 (0%)
More than 60 days	5 (6.25%)	1 (1.25%)

*No statistical test was applied considering "0" subjects in one of the cells.

Among the Swab culture group, 39 (48.75%) showed timing of granulation tissue formation between 15 to 30 days. The number of cases with granulation tissue formation between 31 to 45 days, 46 to 60 days, and more than 60 days was 21 (26.25%), 15 (18.75%), and 5 (6.25%), respectively. In the tissue culture group, the proportion of timing of granulation tissue formation between 15 to 30 days, 31 to 45 days, and more than 60 days was 46 (57.5%), 33 (41.25%), and 1 (1.25%), respectively (**Table 5 Figure 2**).

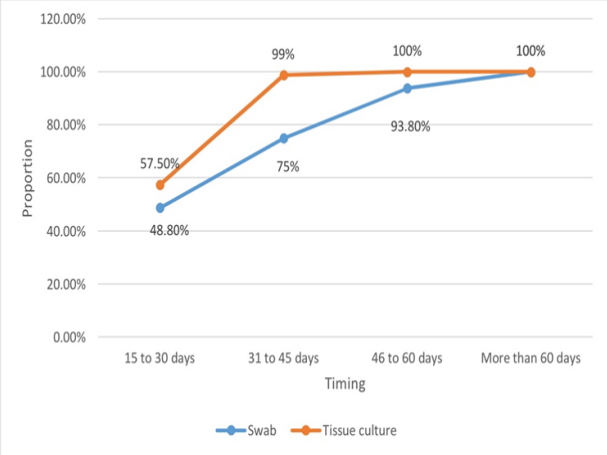

Figure 2 Cumulative percentage of timing of developing granulation tissue in both the study groups (N=16)

DISCUSSION

Diabetes Mellitus is rapidly becoming a major global public health concern, carrying a significant burden of morbidity and mortality. Among developing countries, the prevalence of diabetes has notably increased, with China and India leading the trend. The complications of diabetes encompass microvascular and macrovascular diseases, with diabetic foot ulcers being a major microvascular complication.

Diabetic foot ulcers arise primarily from Diabetic Neuropathy, a microvascular complication of diabetes, among other factors. It is a significant contributor to nontraumatic lower extremity amputations, with 5% of diabetics developing foot ulcers annually and 1% requiring amputation. Microbiological investigations play a crucial role in determining the treatment approach and the choice of antibiotics. In recent times, clinicians rely more on microbiological data than clinical data for diagnosing infections. Samples from diabetic foot ulcers are obtained through tissue biopsy or wound swab.

Our study, conducted at SRM Medical College, Hospital, and Research Centre from June 2016 to June 2017, focused on 160 subjects and compared tissue culture with swab culture in the context of diabetic foot ulcers. The primary outcome variable was the timing of granulation tissue appearance, with the primary explanatory variable being the sample collection method for culture—either Swab or Tissue culture.

Sociodemographic Profile:

The baseline sociodemographic variables such as age and gender were comparable between the two groups in our study. We observed a male predominance, consistent with the general demographic profile of diabetes. However, gender and age were not significant factors affecting the concordance between swab and tissue culture sensitivity.

Clinical Profile:

There was no statistically significant difference between the swab culture and tissue culture groups concerning clinical profiles like HbA1c and baseline area of the ulcer [15][16]. Both groups were comparable in terms of baseline clinical characteristics.

In our study, 76.88% of swab cultures and 92.50% of tissue cultures were positive for organisms. Tissue culture identified more organism-positive samples than swab culture, aligning with findings from other studies [12][13][14][17].

The most common organisms isolated in swab culture were Proteus (14.38%), Pseudomonas (11.88%), E. coli (8.13%), and Acinetobacter (8.13%). In tissue culture, Pseudomonas (16.88%), Proteus (14.38%), Klebsiella Pneumoniae (13.13%), Staphylococcus aureus (10.63%), and E.coli (10.63%) were the prevalent organisms.

The study explored an additional aspect by investigating the time for the development of granulation tissue in the two groups. The timing of granulation tissue appearance was faster in the group treated based on tissue culture compared to swab culture. The proportion of people who developed granulation tissue within 30 days was higher in the tissue culture group.

CONCLUSION

- No statistically significant differences were observed in both study groups concerning age, gender composition, duration of diabetes, baseline area of the ulcer, and glycaemic control.
- Swab culture was positive in 76.88% of the population, and tissue culture was positive in 92.50% of the study population.
- The most common organism isolated in swab culture was Proteus (14.38%), followed by Pseudomonas (11.88%), E. coli (8.13%), and Acinetobacter (8.13%).
- Pseudomonas was the most common organism isolated in tissue culture in 16.88% of the subjects. The other common organisms isolated were Proteus, Klebsiella Pneumoniae, Staphylococcus aureus, and E.coli in 14.38%, 13.13%, 10.63%, and 10.63%, respectively.
- Mixed organisms were isolated in 23.22% of swab cultures and 4.87% of tissue culture specimens.
- The cumulative proportion of subjects developing granulation tissue was faster in the tissue culture group.

Strengths: The study compared the rate of healing of ulcers when patients were treated based on swab culture compared to tissue culture, which is of high practical importance in resource-poor settings.

Limitations:

1. The study used the time window of granulation tissue appearance as the primary outcome measure, and more specific outcome measures like the rate of reduction in ulcer size could have been more appropriate.
2. The study's baseline balance between the two groups was limited to a few potential confounding factors, and the role of potential confounding by other relevant variables could not be completely ruled out.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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