



## STUDY ON SWAB CULTURE VS DEEP TISSUE BIOPSY CULTURE IN CHRONIC WOUND INFECTIONS

Dr, Selwyn J.Kumar<sup>1</sup>, Prof. Dr. J. A. Jayalal<sup>2</sup> and Dr.Bhagavadh<sup>3</sup>

<sup>1,3</sup>Assistant Professors Department of Surgery, Kanyakumari Medical College Under Dr. MGR. Medical University

<sup>2</sup>Professor of Surgery, Department of Surgery, Kanyakumari Medical College under Dr. MGR. Medical University.

**\*Corresponding Author: Jayalal Johnrose**

Professor of Surgery, Department of Surgery, Kanyakumari Medical College under Dr. MGR. Medical University.

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### ABSTRACT

**Objectives:** Aim of this study is to compare the swab cultures with deep tissue biopsy cultures taken from the same wounds of diabetic and non diabetic patients with chronic infections. **Materials and methods:** To compare the two culture methods, prospective study was conducted in 100 patients admitted with chronic non healing ulcer, in the surgical septic ward in Kanyakumari Government Medical College Hospital between March 2016 and December 2016. The patients were divided in to two groups one with patients having diabetes and the other with non diabetic patients. Both swab and deep tissue biopsy taken from the wounds of these patients and culture and sensitivity was done on these samples and the results were analysed. **Conclusion:** Both swab and deep tissue biopsy techniques have the same results in providing the more comprehensive description of wound flora of both diabetic and non-diabetic patients in grade 2 and 3 Wagner grade ulcers and in grade 4 and grade 5 ulcer the deep tissue biopsy will give more specific data.. Infection by Klebsiella and Pseudomonas were the commonest pathogens. Both the culture method appears valid as long as the wound is cleansed thoroughly and necrotic material is removed prior to culture collection. It is reasonable to use the swab-based culture method for chronic non healing wounds.

**KEYWORDS:** Chronic Wound Infection, Deep Tissue Culture, Swab Culture, Wound Healing.

### 1. INTRODUCTION

A wound is called 'Chronic' when it does not heal within its normal healing period and retained in any one or more of the Phases of wound healing for more than three months.<sup>[1]</sup> The various causes for the chronicity of the wounds include ischaemia, neuropathy, infection, poor general condition, systemic illness, prolonged immobilization etc. Wound infection means multiplication of microorganisms within the wound leading to prolonged and or excessive inflammatory response, delayed collagen synthesis, delay in epithelialisation and resulting in tissue damage.<sup>[2]</sup>

Chronic wound infections can lead to increased morbidity and mortality, prolonged hospitalization, amputations, increased medical expenditure and financial loss to the family. Patients with chronic wounds develop infections with bacteria having resistance to antibiotics. In addition, the wounds become chronic when the patients carry drug resistant bacterial strains such as methicillin-resistant Staphylococcus aureus (MRSA) in their wounds.<sup>[3]</sup>

Identification of the infections in wounds is very difficult and it is usually assessed clinically based on the symptoms and signs. It is more difficult to diagnose a chronic wound infection than acute wound infection since in the former, the signs and symptoms are often more subtle. So in a patient with suspected wound infection, cultures are important in diagnosing the infection, identifying the infecting organism, determining the number of organisms present and the antibiotic sensitivity. A wound is said to be infected if the microbial growth in the wound is greater than  $10^5$  colony-forming units (CFUs)/gm of tissue or ml of wound fluid.

The wound are graded depending upon the Wagner grading based on the depth involved and associated infection on the deeper structures.

#### Wagner Grading System

Grade 1: Superficial Diabetic Ulcer.

Grade 2: Ulcer extension-Involves ligament, tendon, joint capsule or fascia without abscess or osteomyelitis.

Grade 3: Deep ulcer with abscess or Osteomyelitis.

Grade 4: Gangrene localized in a portion of forefoot.

Grade 5: Extensive gangrene of foot.

The bacterial infection in a wound is best determined by quantitative culturing and identifying the sensitivity of the organism to the antibiotics if grown, in culture of wound swab, tissue biopsy, wound fluid obtained after curettage, or fine-needle aspiration.

Many researchers found that swab technique may not identify the exact organisms present in the wounds when compared to deep tissue biopsy. Furthermore, these studies show great difference in their results, which shows relation between swab and tissue specimens varied from 9% to 62%.<sup>[4,5]</sup>

This study is aimed to compare the results obtained from the swab and tissue cultures, using swab and tissue biopsy samples collected from the same chronic wound of diabetic and non diabetic patients.

## 2. MATERIALS AND METHODS

The study was done as a prospective study. The study was conducted in 100 patients, both diabetic and non diabetic patients with chronic non healing ulcer admitted in the surgical septic ward in Kanyakumari Government Medical College Hospital between March 2016 and December 2016. Swab and tissue cultures were taken from the same wound of these patients, who haven't taken any systemic or topical antibiotics for at least 1 week.

### 2.1: Ethical Aspects

This study has been approved by the Ethical Committee of Kanyakumari Government Medical College. From all the patients included in the study a written informed consent was obtained after explaining about the methods of taking cultures, possible complications while taking tissue for culture and the benefits of conducting the study.

### 2.2: Inclusion criteria

All the patients admitted with chronic wounds due to diabetic ulcer, venous ulcer, pressure sores and non healing traumatic ulcers.

### 2.3: Exclusion criteria

1. Wounds with gangrene and suspicion of gas gangrene.
2. Perianal wounds with faecal contamination.
3. Very ill patients.
4. Patients not willing to participate in the study.

### 2.4: Method

In the patients admitted with chronic non healing ulcers which were included in the study, initial debridement was done in needy patients and daily dressings were done using saline, hydrogen peroxide and/or povidone iodine solution. These patients were not given any systemic or topical antibiotics till the culture is taken. The culture has been obtained from the properly cleaned and prepared wound tissue in order to obtain culture free of surface contamination.

Culture and sensitivity was done from the viable wound bed using swab using Levine technique and deep tissue punch biopsy techniques from the same wounds of these patients at the same time. The tissue sample taken from the wound was immediately put into a sterile bottle and sterile saline was poured into the bottle for suspension of the tissue. Both the swab and the tissue biopsy bottles were then transported to the Microbiology laboratory. From these identification of the pathogen was done by standard culture methods. Antibiotic susceptibility pattern of isolates were determined using standard methods.

### 2.5: Statistical analysis

The results were tabulated and Statistical analysis was performed with Statistical Package for Social Sciences.

## 3. RESULTS

### 3.1: Age and sex distribution

The profile of the patients and their diabetic status were analyzed and data tabulated in Table 1 and Figure 1. Majority of 68% of patients were with chronic wound infection were having Diabetic and 32% were non diabetic and this is statistically significant  $p < .001$ . 64% of our patients were in the age group of 51-70 and followed by 18% of our patients were in the 31 to 50 age group. 76% of patients with chronic wound infection were male and male: female ratio is 7.6:2.4.

Table: Age, sex and comorbid status distribution.

AGE	DIABETIC			NON DIABETIC		
	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL
< 30 years	0	0	0	2	1	3
31-50 years	7	6	13	4	1	5
51-70 years	28	17	45*	12	7	19*
>70 years	9	1	10	5	-	5
Total	44	24	68	23	9	32

Statistically significant  $p < .001$ .

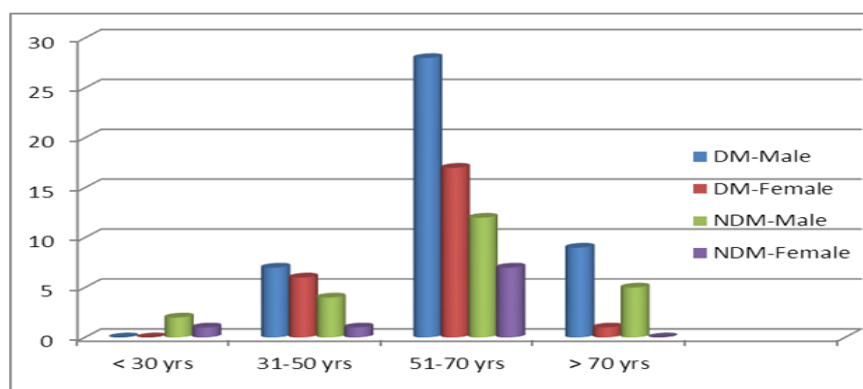


Figure 1: Age and Diabetic and Non diabetic variables.

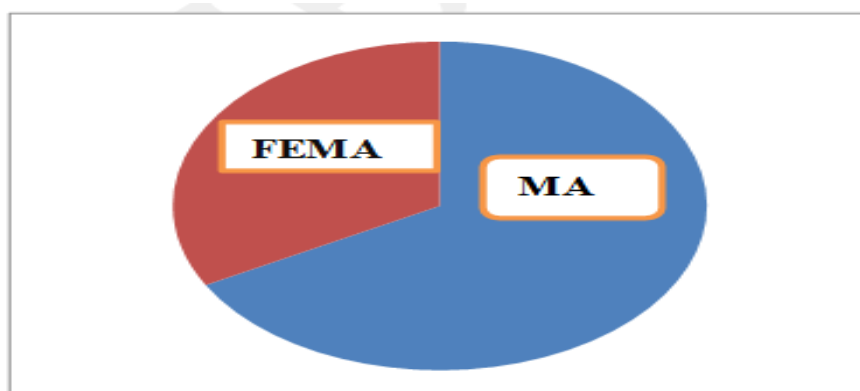


Figure 2: Male vs female with chronic wound.

**3.2 Analysis of grade of the ulcer and concordance of swab vs tissue culture:** As per the Wagner grading system the ulcer were grouped and data tabulated in Table 2. In Grade 2 ulcer both swab culture and tissue

culture yielded identical and positive culture. However in deeper ulcers of grade 3 and grade tissue culture yielded more positive results. The data are tabulated in table 3.

Table 3: Frequency of distribution of grades and positive culture reports.

Wagner Grade	Number of patients	Positive swab culture	Positive tissue culture
Grade 1	14	13	2
Grade 2	61	55	53
Grade 3	12	10	10
Grade 4	8	5	7
Grade 5	5	3	3
Total	100	86	75

### 3.3 Analysis of growth of bacteria in cultures

The culture reports of each patient on both swab culture and deep tissue culture were received, tabulated and analyzed. 52% showed positive culture in both the culture methods and 17% of patients showed no growth

in both the culture. However in 22% of patients the swab culture was positive but the deep tissue biopsy were negative and in only 1% of patient the swab culture was negative and tissue culture showed the growth of the organism. The data are tabulated in table 4.

	DM n=68	NON DM n=32	TOTAL n=100
No growth in both culture	12	5	17
No growth in swab c/s Growth in tissue c/s	-	1	1
Growth in swab c/s No growth in tissue c/s	17	5	22
Same organism in both culture	34	18	52
Different organisms in both	5	3	8
Total	68	32	100

**Table 5: concordance of swab versus tissue culture.**

ORGANISM	DM		NON DM		TOTAL	
	swab C/S	TISSUE C/S	swab C/S	TISSUE C/S	swab C/S	TISSUE C/S
No growth	10	17	4	8	14	25
Enterococcus	2	0	0	0	2	0
Staphylococcus aureus	6	6	2	2	8	8
Klebsiella species	12	10	6	7	18	17
Escherichia coli	8	7	4	3	12	10
Citrobacter species	2	0	2	2	4	2
Pseudomonas aeruginosa	16	15	7	6	23	21
Proteus mirabilis	8	11	5	4	13	15
Acinetobacter species	2	0	1	0	3	0
Coagulase negative S.aureus	2	2	1	0	3	2
Total	68	68	32	32	100	100

In both swab and tissue culture 18% and 17% of patients had the growth of Klebsiella and 23% and 21% ulcers shown pseudomonas growth. No growth was reported in 14% of swab culture and 25% of tissue culture as majority of grade 1 ulcer shown no growth in tissue culture.

#### 4. DISCUSSION

Identifying the pathogenic organism in an infected wound is the mandatory requirement for executing effective treatment protocols. A reliable and easily reproducible sampling technique is ideal to identify pathogens growing in infected diabetic foot wounds. In general, three methods of culture techniques are commonly used.

- Levine technique swab culture
- Z tract or 10 point swab culture
- Deep tissue punch biopsy culture

A systematic review of diagnosis of infections in diabetic foot ulcers has concluded that the available evidence is too weak to determine the optimal sampling technique.<sup>[6]</sup> Though there are many guidelines exist for obtaining a culture, no single guideline is used universally. However, irrespective of the technique used, it is mandatory that certain basic principles are to be followed:

- The ulcer debridement and clean lavage prior to the process of taking culture
- Culture must be collected prior to the use of topical or systemic antibiotics.
- Central necrosis and nonviable portions are common in an infected ulcer and culture should be taken only from the viable area.
- Cross contamination and secondary infections are the common mistakes. Adequate sterile precautions must be observed both by the surgeon and preserving culture bottles.

#### Swab culture

In clinical settings, a swab culture is the most common technique used because it's practical, noninvasive, and cost effective. If done properly, it usually identifies the bacterial species of the infection and helps guide antibiotic therapy.

In Levine technique, after observing sterile protocol, the wound is debrided and washed with saline. The cotton swab is applied over the viable part of the wound, over an area of 1 cm<sup>2</sup> and gentle pressure is applied over the

wound to the extent of causing minimal bleed for 5 seconds, thereby the fluids containing organisms will also ooze out and will be get adherent to the cotton swab.

In Z tract or 10 point swab culture instead of from one particular area, the swab is taken in 10 different points at the stroke line of Z across the ulcer. In other words, the Z-technique involves rotating the swab in a zigzag fashion covering the entire injured area across the wound, without touching the wound edges.

Angel ED, et al. has reported the superiority of Levine's technique over the Z technique.<sup>[7]</sup> In our study, the Levine technique is followed in taking all swab culture.

#### Deep tissue culture

A deep-tissue biopsy using a punch biopsy forceps or ordinary Allis forceps is commonly used for a quantitative culture. A quantitative culture can determine the colony counts per gram of tissue. It is the gold standard for identifying wound bio burden and quantifying the infections and infective organism. A deep-tissue biopsy after initial debridement and cleaning of superficial debris with normal saline solution is the most useful way to detect invasive organisms.<sup>[8]</sup>

Many studies reported by researchers consider that tissue biopsy is the best method for the identification of pathogens in chronic wounds, especially the Diabetic foot ulcers, because deep biopsy is not prone to superficial contamination.<sup>[10][11]</sup> K. Gjødtsbøl et al. has indicated that there is no need for biopsy, as there are no significant differences in the bacterial species isolated between swab and tissue samples.<sup>[12]</sup>

Drinka et al. who compared swab specimens to biopsies and found 2.34 isolates per patient using a swab and 2.07 with a biopsy in 29 severe diabetic foot ulcers and argued that taking a swab or biopsy may be equally reliable.

Time requirements, expert technician processing, and the introduction of a trauma have made tissue biopsy not popular for both the patient and the professional.

Bonham et al has also concluded a maximal concordance of 62-72% in results between swab and biopsy.<sup>[13]</sup>

In our study in the Grade 2 and 3 ulcer which constitute 73% both the swab and tissue culture has yielded the same positivity and in 90% of the organisms isolated are also identical. However in the Grade 4 and 5 ulcers more organisms are isolated in tissue culture and in grade 1 ulcer swab culture is the best.

Our study revealed that the consistency of the microbiological results between the two sampling techniques decreased as the Wagner infection grade increased. A total of 91.0% of the patients with grade 2 and 3 wounds would have been treated with antibiotics adequately based on the swab culture results alone. However, only 41.4% of those with grade 4 wounds and 41.2% of those with grade 5 wounds would have been adequately treated. In addition, the proportion of patients who may have been treated inadequately based on the swab culture results alone increased from 1/13 (9.0%) of those with grade 2 and 3 wounds to 19/31 (58.6%) of those with grade 4 wounds and 9/12 (58.8%) of those with grade 5 wounds. Hence, for grade 2 and 3 wounds, swabbing, is a easier to perform and a relatively noninvasive, satisfactory clinical sampling technique compared with deep tissue biopsy, which has high risk of injury to surrounding tissues, blood vessels, and nerves. However, our data have demonstrated that it is necessary to perform tissue biopsy to obtain an accurate microbiological diagnosis of chronic ulcers to guide clinicians in choosing an appropriate antibiotic therapy for wounds of grade  $\geq 3$ .

## CONCLUSION

From our study it is evident that both the swab and deep tissue biopsy techniques have the same results in providing the more comprehensive description of wound flora of both diabetic and non-diabetic patients in grade 2 and grade 3 ulcers, In addition, our results do not directly suggest that deep tissue cultures are superior. Both the culture methods appears valid as long as the wound is cleansed thoroughly and necrotic material is removed prior to culture collection. Based upon this analysis, we believe that it is reasonable to use the swab-based culture method for chronic non healing wounds in grade 2 and 3 ulcers and in grade 4 and 5 ulcers the deep tissue biopsy will be good to give more accurate data.

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