Research Article

The role of quantitative bacteriology on burn wound management

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ABSTRACT

Background: Severe burn wound infection and sepsis is associated with high mortality. Early diagnosis of infection or sepsis is of utmost importance on burn wound management. Evaluation of the burn wound by a surface swabbing has been the principle method in most of the institutions. This study emphasizes a method of quantitative bacterial count by a tissue biopsy technique for a more accurate way of assessing the burn wounds. Surface swabbing gives surface colonization while a count of 10⁵ CFU/gm. of tissue in the quantitative assay predicts sepsis, skin graft survival and burn wound healing.

Methods: 83 surface swabs and 246 tissue biopsy specimens were collected from a total number of 83 patients. Tissue biopsy was taken, weighed and homogenized in a lab blender stomacher 80. Four fold dilutions of the homogenate were made. Then they were inoculated in MacConkey and blood agar plates and colonies were counted. Statistical analysis used: SPSS version.

Results: Out of 83 patients, 54.22% were females and 45.78% were males. The most common age group affected was 16-30 years (51.81%) and 31.33% acquired 10-20% TBSA burns. Bacterial counts of <10⁵ yielded a graft take of 95-100%, while graft loss occurred with bacterial counts of >10⁵.

Conclusions: Mortality due to acute burn infections is about 5%. Quantitative bacteriology by the above method is simple and provides an exact bacterial count and reliable data in predicting burn wound sepsis. This should be employed in all centres to reduce burn related deaths due to sepsis.

Keywords: Burns, Quantitative bacteriology, Sepsis, Mortality

INTRODUCTION

Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality. Infection causes 50% to 60% of deaths in burn patients in spite of intensive therapy with antibiotics.¹ Burn wounds are highly susceptible to colonization and in order to promote infection control in a burn patient, burn wounds should be recognized at sites of microbial colonization which may progress to invasion which leads to systemic dissemination, if not prevented.

Colonization arises from the patient's own resident and transient flora.²⁻⁶ Following burn injury, the sub eschar tissue is the site where bacterial proliferation takes place and the bacteria proliferate here and reach levels greater than 10⁵ bacteria per gram of tissue. When the level of bacterial growth exceeds 10⁶ or 10⁷, then microbial invasion into the blood stream occurs. This is the most important cause of septicemia.⁷

Multiple studies from the United States army institute of surgical research (USAISR) proved invasive burn sepsis synonymous with a bacterial count of 10⁵ or greater per gram of tissue. So monitoring the bacterial load has

become an important tool in predicting and preventing invasive burn wound sepsis.⁸⁻¹⁰

Nearly 11 million people worldwide are burned severely enough to require medical attention annually, according to WHO estimate of 2004. More women worldwide are severely burned each year. According to new 2010 global burden of disease study, burns remain a leading cause of morbidity and mortality. Burns also cause more than 19 million DALYs (Disability Adjusted Life Years), productive year lost to being disabled from a disease or injury.

In contrast to the developed world, the burn load in developing countries is many folds higher. As per the report of the Indian national crime records bureau (INCRB) of 2007, out of 340,794 total accidental deaths, 20,772 (6.2%) were due to fire accidents. There were 10,391 (8.5%) deaths classified as suicidal burn deaths out of a total of 122,637 suicidal deaths. The mortality due to burn injuries was reported as 3.5 per 100,000 population.¹¹

For monitoring of burn wounds the three most popular methods are surface swabbing, blood cultures and quantitative bacterial assay by deep tissue biopsy techniques. The value of superficial cultures in wound assessment has been questioned and it may fail to yield growth whereas biopsied tissue may yield significant numbers of bacteria. Swab sampling has been challenged on the basis that the superficial colonizers do not reflect the organisms of deeper tissue and that subsequent cultures do not correlate with the presence of pathogenic bacteria. But the procedure of superficial swab samples is simple, inexpensive, non-invasive and convenient for the majority of wounds. On the other hand, blood cultures yield a positive culture report only at a later date even when the organisms are present.¹²⁻¹⁴

Surface wound swabs currently used in many centers in India do not give the exact count of pathogens involved in burn wound sepsis. Therefore in order to monitor burn wound, quantitative bacteriology is essential and there should be a technique for monitoring bacterial counts. This study emphasizes a method of quantitative bacterial count by a tissue biopsy technique for a more accurate method of assessing the burn wounds. Surface swabbing gives surface colonization and may not accurately reflect the organism causing wound infection while a count of 10⁵ CFU/gm of tissue in the quantitative assay are likely to develop sepsis and also to predict graft bed receptiveness and safety of wound closure.^{15,16} An objective of this study was to emphasize a method of quantitative bacterial assay by tissue biopsy technique for an accurate microbial assessment of burn wound infections and to emphasize the importance of bacterial assay to provide clinical guidelines for reconstructive procedures and to assess the clinical outcome of patients who underwent reconstructive procedures following

guidance of quantitative bacterial assay and to predict sepsis by this method and thus aiding its prevention.

METHODS

A total number of 246 tissue biopsy samples were collected from 83 patients with burns wound. Samples from the burn wounds were collected by both surface swabbing and by tissue biopsy technique. To obtain a culture of burn surface, topical agents were first removed with gauze soaked in sterile saline. The method of collection was deep swabbing, or aspiration of the bleb and by two sterile swab sticks. One swab was used for direct gram staining and the other swab was inoculated in MacConkey and blood agar plates and incubated for 18 to 24 hours at 37°C. At the end of the incubation period, the plates were examined for the isolates.

For quantitative cultures of biopsy samples, the wound was washed with saline soaked gauze pads to wash off the topical agents. The wound was biopsied by making two parallel incisions with surgical 11 blades, approximately 1 to 2 cm in length and 0.5 cm apart. Then the tissue was elevated by Alleys' Forceps and cut to a sufficient depth to obtain a small portion of the healthy underlying fat 16 the tissue was placed directly into a sterilized pre-weighed homogenizer bag containing 1ml normal saline and the bag was re-weighed. Weight of the tissue was obtained by subtracting the first weight from the second by the formula.

$$(C = B - A)$$

Where, C = weight of the tissue, B = weight of bag with saline and tissue, A = weight of bag with saline.

The tissue placed in a homogenizer bag with 1ml of normal saline was homogenized in a tissue homogenizer (Lab Blender stomacher 80) for 30 seconds. Then with the homogenate, gram staining and quantitative bacteriology were performed simultaneously.

Figure 1: Lab Blender stomacher 80.

Several dilutions were made with the homogenate. Four test tubes containing 4.5 ml of normal saline each was taken. 0.5 ml of the homogenate was taken from the homogenizer bag with the help of a micro pipette and added to the first test tube containing 4.5 ml of normal saline. With a new micropipette 0.5 ml was transferred to

the second tube, mixed well and 0.5 ml was transferred to the third tube, mixed well and added to the fourth tube and then 0.5 ml taken from the fourth tube was discarded. Thus four dilutions 1/10, 1/100, 1/1000, 1/10000 were made (Figure 1, 1A).

Figure 1 (A): Processing of the tissue biopsy specimen.

First direct gram staining was done from the homogenate fluid. MacConkey agar plates and blood agar plates were kept ready for inoculation. Each plate was divided into four quadrants and labeled as 1/10, 1/100, 1/1000, 1/10000. 0.01ml of homogenate was taken from each dilution tube and dropped in the MacConkey and blood agar plates from a height of 2.5cm. Plates were then placed in the incubator with the lids slightly open for 15 minutes and then closed and incubated for 24 hours at 37°C. The number of colonies was counted by a hand lens (Figures 1B, 1C).

Figure 1 (B): Homogenate in dilution tube.

The colony count per gram of tissue was obtained by the formula of Miles and Misra²³

$$(\text{CFU/gm of tissue} = C \times D \times V/W \times 0.01)$$

Where, C = the total number of Colony forming units, D = the dilution factor, W = the weight of the tissue, V = the volume of normal saline, 0.01 = the volume of the inoculum.

Bacterial isolates were identified by adopting the procedures of Gram staining, motility and routine biochemical reactions (Figures 2, 2A, 2B).

Figure 1 (C): Homogenate transferred to MacConkey and blood agar plates.

Figure 2: Colonies of *Klebsiella pneumonia*.

These methods of quantitative cultures were initially developed by Loebl. He collected the tissue sample by making two parallel incisions and by means of sterile tissue forceps elevated and biopsied the sample by means of a scalpel. Then he macerated the tissue and made several dilutions. Lawrence described an open method.

Figure 2 (A): Colonies of *Pseudomonas aeruginosa*.

Figure 2 (B): Colonies of *Staphylococcus aureus*.

Baxter homogenized the tissue with the help of knife after suspending it in 1-2 ml of normal saline. Homogenization can also be done with a mortar and pastel. Robson et al used a polytron homogenizer. Ganatra et al used a punch biopsy forceps followed by homogenization in a Lab Blender Stomacher. Buchanan et al used a sterile scalpel blade and forceps and cut into small pieces and homogenization carried out in a tenbroeck tissue grinder.¹⁷⁻²²

In this study a method similar to that described by Loebel was adopted with one difference in that, Lab Bender Stomacher 80 had been used which ensured sterile and safe method. Statistical analysis; all statistical analysis were performed using SPSS version with (p value) and $p < 0.05$ was accepted as statistically significant.

RESULTS

Out of 83 cases, the most common age group affected was 16-30 years (51.81%), followed by the age group 31-45 years (27.71%), 45 cases were females (54.22%) and 38 cases were males (45.78%), 26 persons were between 10-20% TBSA burns (31.33%) followed by 18 cases between 21-30% TBSA burns (21.69%), 35 cases had first degree burns (42.17%) followed by 26 cases who had third degree burns (31.32%). In the tissue biopsy specimens, out of 83 samples taken on Day 1, 74 samples were culture positive (89.16%), out of 70 samples taken on Day 4, 69 samples were culture positive (98.57%) and out of 56 samples taken on Day 9, 56 samples were culture positive (100%) (Figures 3, 4).

Figure 3: Day wise culture results in tissue biopsy specimen.

Figure 4: Tissue biopsy single and multiple isolates.

Table 1: Showing the skin graft uptake after assessing quantitative bacteriology.

Age	Gender		Raw area %	Isolate	Bacterial count	Clinical intervention	Graft uptake
	M	F					
30		F	20	Staphylococcus aureus	5.09	SSG	60%
16	M		20	Staphylococcus aureus	5.34	SSG	50%
50	M		25	Pseudomonas aeruginosa	0.51	SSG	70%
20	M		20	Klebsiella pneumoniae	0.30	SSG	95%
48	M		30	-	NG	SSG	95%
19	M		10	Pseudomonas aeruginosa	Nil	SSG	70%
48	M		30	-	NG	SSG	100%
50		F	30	Klebsiella pneumoniae	0.54	SSG	95%
18		F	20	Staphylococcus aureus	6.54	SSG	60%
18		F	20	Pseudomonas aeruginosa	1.80	SSG	60%
22	M		30	-	NG	SSG	100%
60	M		15	Staphylococcus aureus	4.13	SSG	50%
Mean	33.25		22.5		2.02		
SD	16.48		6.57		2.19		
SEM	4.76		1.89		0.632		

While comparing both the swab and tissue cultures, both showed predominance of *Staphylococcus aureus*. It should be noted that CoNS, *Micrococci*, *Diphtheroids*

and culture negatives were reported only in surface swabbing but not in tissue biopsy cultures. Moreover, *Klebsiella oxytoca* and *Escherichia coli* were more

common colonizers in the tissue biopsy cultures. Out of the 294 isolates, *Staphylococcus aureus* (96) showed a mean bacterial count of 4.40×10^5 , *Pseudomonas aeruginosa* (38) was 3.57×10^5 . *Escherichia coli* (52) and *Klebsiella oxytoca* (6) showed mean bacterial counts of 0.89 and 0.54 respectively which was less than 105 CFU/gm of tissue (Figures 5, 6 and 7).

Figure 5: Distribution of swab culture isolates.

Figure 6: Distribution of tissue biopsy isolates.

Figure 7: Comparison of swab and tissue biopsy isolates.

In the characteristic analysis, burn depth showed no significant association with the results of quantitative biopsy cultures. As for gender, there was significant association between the positive biopsy results and the female gender ($p < 0.05$). Age group showed a significant association with the results of biopsy cultures with the age group between 16-30 ($p < 0.05$). There was

significantly lower level of TBSA in patients with positive surface swab cultures compared with that biopsy cultures ($p < 0.05$) (Figures 7A, 7B and 7C).

Figure 7 (A): Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* (%).

Figure 7 (B): Antimicrobial susceptibility pattern of *Acinetobacter baumannii* (%).

Figure 7 (C): Antimicrobial susceptibility pattern of *Citrobacter freundii* (%).

Out of 83 patients, this technique was employed in 12 burn patients whose raw areas were clinically ready for receiving skin graft. Tissue biopsy cultures and swab cultures were performed three days prior to the grafting procedures. Out of 12 patients, 8 patients were males and 4 patients were females. Their age varied from 16 years to 60 years and was in the mean age group of 33-50. Their total body surface areas burnt varied from 10% to 30% with a mean raw area between 22-50. Their bacterial

counts ranged from 0.30×10^5 to 6.54×10^5 with a mean bacterial count of 2.18. *Staphylococcus aureus* was isolated in 4 patients and showed the highest bacterial counts. *Pseudomonas aeruginosa* was isolated in 3 patients and the bacterial counts were ranged from 0.51 to 1.80. *Klebsiella pneumoniae* was isolated in 2 patients and their counts were ranged from 0.30 to 0.54. Split skin graft was done for all the patients. The “graft take” for all the 12 patients were observed. Patients with bacterial counts ranging from 1.80 to 5.34×10^5 had a “graft take” ranging from 50% to 60%. Patients with bacterial counts from 0.30 to 0.51×10^5 had a “graft take” of 70% to 95%. Patients who were culture negative had a 95% to 100% “graft take” Figures 8, 8A, 8B, 8C.

Figure 8: Antimicrobial susceptibility pattern of *Escherichia coli* (%).

Figure 8A: Antimicrobial susceptibility pattern of *Proteus mirabilis* (%).

Figure 8 (B): Antimicrobial susceptibility pattern of *Klebsiella pneumoniae* (%).

Figure 8 (C): Antimicrobial susceptibility pattern of *Methicillin resistant staphylococcus aureus* (%).

DISCUSSION

Infection in the burn wound continues to be the main cause of morbidity and mortality in patients who are admitted to hospital with major thermal burns.¹ Burns provide a suitable site for bacterial multiplication and more persistent richer sources of infection mainly because of larger area of involvement and longer duration of stay of the patients in the hospital.²³ Quantitative bacterial assay had been employed in this study to effectively monitor the burn wounds and to predict and prevent invasive burn wound sepsis at an early stage. Tissue biopsy samples had been taken and quantitative bacteriology was performed and also quantitative bacterial assay was compared with that of surface swabbing as it is the chief modality of investigation in most of the tertiary care centers.

Tissue biopsy and quantitative bacterial count of verification of microbial invasion into viable unburned tissue have been the “gold standard” to confirm invasive burn wound infection.²³ This method is the most beneficial in determining the optimal time for skin grafting and surgical wound closure.²⁴ However, the value of a single biopsy specimen particularly in slow healing chronic wounds is debatable. So multiple specimens are taken for a more accurate diagnosis.

Bacteriological assessment is done on the day of admission, as it is important to know the bacteriological status at the commencement of treatment, on the third or fourth day, when the period of maximum exudation is over to assess the bacterial colonization, twice weekly as there is an indication, according to clinical signs, three days before any proposed grafting procedure, so that the results are available before making a final decision about the surgery.²³

Burned patients do not have same type of injury so that the severity and extent of the burn injury vary greatly from patient to patient. Many studies were done using various sampling techniques and microbiological methods, and comparative studies were done before the advent of early excision therapy.^{1,21,22} Steer and colleagues, had reported the recent largest studies and

compared the results of biopsy cultures and surface swabs. In their initial study qualitative and quantitative bacterial counts were compared. Although there was a significant correlation between the bacterial counts obtained by biopsy and swab, the counts obtained by one method were poorly predictive of the counts obtained by the other. Also, parallel cultures taken on multiple occasions showed a significant correlation between bacterial counts obtained from two biopsies or two swabs simultaneously, but there was wide variation in bacterial densities from the same burn wound at the same time. The study concluded that the use of quantitative assay in burns was limited by the unreliability of a single surface swab or biopsy sample to represent the whole burn wound.

Steer and coworkers in their work studied a relationship between bacterial counts collected by surface swabs and burn wound biopsy cultures. They collected both the specimens either immediately prior to excision and grafting or during routine dressing changes. From their study they demonstrated that quantitative bacteriology by burn wound biopsy or surface swab did not aid in the prediction of sepsis or graft loss.

Loebl and colleagues demonstrated that the microorganisms obtained from the burn wound surface which was not excised showed poor correlation with that of tissue biopsy samples taken from the sub-eschar tissue. Freshwater and Su observed that the quantitative burn wound cultures should be clinically correlated with the burn wound infection and reported accordingly so that it served as a useful guide in the management of burns patients with large TBSA burns. Tahlan and colleagues also compared surface swabs and the burn wound biopsy cultures in their study in second and third degree burns. They did not observe any difference in the isolates from the swabs and wound biopsies.

For routine surveillance of the burn wound infections the apt sampling technique should be adopted according to the area of the burn wound. Superficial swab proves to be a most convenient and least invasive. Moreover swabs can be taken from areas where skin is very thin to be biopsied like ears, eyes and phalanges. Deidre church and colleagues viewed that tissue biopsy samples should be sent for quantitative culture from burn wound in patients with sepsis. But a coordinated approach using quantitative tissue biopsy, blood, and urine samples can provide a best approach in assessing the burn patients with sepsis.^{24,25}

So the main aim for any strategy dealing with burn wounds should have definite goals in achieving an effective infection control. The goals should be to prevent transmission of exogenous organisms to patients or personnel, to control the transmission of endogenous organism, that is, the normal flora to sites at increased risk of infection and to protect and support existing defense's in patients with seriously impaired resistance.

CONCLUSION

Comparison of the tissue biopsy with that of surface swabbing proved that quantitative bacteriology reflected the exact microbial load in the subeschar tissue. It proved very useful in surgical intervention by reduction of the microbial load by debridement and skin grafting, provided that the graft bed contains less than 105 bacteria per gram of tissue. Serial quantitative bacterial counts help to alert the clinician about the evolution of colonizers to critical levels and hence plan timely surgical intervention such as escharectomy, consequently preventing the onset of septicemia. This study has proved that quantitative cultures obtained on a routine basis will monitor the progress of bacterial colonization, provide guidance in empirical antibiotic therapy and also allow prompt intervention against bacterial invasion. Here, the method described in this study is simple and easy to adopt. So it should be made as a routine investigative tool in burn care units.

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