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A CLINICAL AND QUANTITATIVE BACTERIOLOGICAL STUDY ON WOUND INFECTION AND EFFECTS OF SOME TOPICAL ANTI-MICROBIAL AGENTS ON WOUND HEALING

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SUMMARY

A total of 165 cases of burns were evaluated by surface swab culture and quantitative biopsy cultures. Staphylococci was the most frequent single organism isolated, although Gram-negatives exceeded the Gram-positives ones. Surface swab culture was found to be a generally reliable indication in 70% of the cases. Framycetin was found to be superior to Povidone iodine and Silver sulphadiazine as far as topical antimicrobial agents are concerned.

(*Key Words*: Burns, Sepsis, Wound Infection)

It is an age old observation that infection delays or prevents healing of a wound. Wound infections may be classified in many ways, such as by the causative organism, the type of injury and the extent of infection. In view of this, the diagnostic yardstick for a true infection of the wound must be agreed upon.

The commonest, simplest, and therefore the most frequently used procedure is taking a swab from the wound for bacteriological culture. The fear often expressed about swab culture is that it may not reflect the true picture of the wound as a whole. Quantitative wound biopsy cultures were therefore recommended in appropriate cases. Understandably such biopsies are not easy to perform routinely in hospitals, thus restricting their usefulness. The present study was aimed at correlating surface swab culture with quantitative wound biopsy culture and also at suggesting a possible critical level of bacterial concentration on wound biopsy which will indicate a true wound infection.

An effort is also made to evaluate the efficacy of some commonly used topical anti-microbial agents with regard to early bacteriological clearance as indicated by surface culture and wound biopsy techniques and whether there exists a significant difference of results between the two techniques as effected by therapy and as correlated with the clinical progress of wound healing.

Materials and Methods

A total of 165 adult patients of either sex presenting with a single open wound caused by burns, trauma, or pressure, were included in the study. Those with abnormally low haemoglobin, abnormal blood counts, low serum protein levels, diabetes mellitus, tuberculosis, malignancy or severe chronic debilitating diseases were excluded.

Wounds were clinically assessed by inspecting the floor, edge, discharge and wound area, and by palpating the margin, wound tissue and base for tenderness. Clinically the wounds were classified into the following two groups :—

(1) *Healthy healing condition* : When there was red florid granulation tissue in the floor, the edge and surrounding skin not inflammed, the edge showed bluish outline of growing epithelium and there was slight or no serous discharge.

(2) *Unhealthy wound condition* : It included two groups (i) Spreading, when there was little or no evidence of granulation tissue in the

floor which was covered with slough, with profuse purulent discharge whose edge and surrounding skin was inflammed, and (ii) Callous, when there was pale smooth granulation tissue in the floor and considerable induration at the base, edge and neighbouring area (Das, 1980).

A tracing of the wound edge was taken after cleaning with normal saline on to a sterile cellophane paper using a needle and methylene blue. Such repeated measurements were used to calculate the reduction in area with treatment.

Bacteriology : One swab and one wound biopsy specimen were collected for qualitative and quantitative culture. The swab was taken after cleaning with normal saline, to remove the topical anti-microbial agent used.

Quantitative culture on wound biopsy specimen was performed as follows : After cleaning the surface of the wound with normal saline, making it free of topical anti-microbials and exudates, biopsy was performed by making two incisions. The specimen was then elevated with tissue forceps and cut from the subcutaneous tissue at a sufficient depth to obtain a small portion of the underlying fat. The specimen obtained in this manner typically weighed 0.02 to 0.05 gm. (Baxter, 1973). This technique produced minor bleeding which could be controlled by digital pressure. Local anaesthesia was not used as these agents are prepared in bacteriostatic solutions which can inhibit bacterial growth in vitro. Biopsy specimens were placed in dry, sterile tubes with the addition of a nutrient broth for transportation to the laboratory. Processing of these specimens was done within one to two hours following their procurement (Baxter, 1973).

Biopsies were processed by weighing each specimen in a sterile pre-weighed petridish on a Torsion semimicrobalance, macerating the tissue specimen with a knife and suspending it on 2 ml. of sterile physiological (0.9%) saline. Serial 1-10 dilutions of this suspension were then prepared in sterile normal saline (Baxter, 1973).

0.1 ml. of each dilution was transferred to a brain heart infusion agar (BHIA) plate, and the inoculum was spread evenly on the surface of the plate with a glass rod (Menon, 1984). Surface viable counts were made after 18 to 20 hours incubation at 37°C by the method of (Miles et al., 1938) and the number of organisms per gram of tissue was calculated. Each colony was assumed to have grown from a single organism in the dilutions (Baxter, 1973). A portion of the specimen was also plated on blood agar and MacConkey's medium, for qualitative analysis. When no growth was obtained in the culture up to 48 hours, it was taken as a negative result.

Treatment Schedule : Each wound was dressed at 24 hour intervals. Patients were randomly divided into four groups (a) Control-sterile paraffin tulle gras dressing (b) Framycetin sulphate cream 1% (Soframycin), (c) Povidone iodine ointment 5%, and (d) Silver sulphadiazine cream 1%.

Follow-up : The wound was examined at each dressing. The reduction in the area of the wound was calculated at weekly intervals for four weeks as also bacteriological examination by the swab and biopsy methods. The clinical condition of the wound was recorded to obtain a correlation with bacteriology. An overall evaluation of treatment was made at the end of the study period.

Results

The mean age of the patients treated was 31.6 years (SE 2.2). The male to female ratio was 106 : 59.

The genesis of wound in the treated patient is shown in (Tab. 1). Trauma was the most frequent cause (62%), followed by burns (38%) and pressure (15%).

The bacterial isolates from the wound and their susceptibility to Framycetin is given in (Tab. 2). Staphylococci were the most frequently encountered organisms followed by E. coli, Pseudomonas, Klebsiella, Streptococci,

Table 1. Genesis of wound in treated patients

Group	Number of patients (Percentage)			
	Burns	Trauma	Pressure sore	Total
Control	8(22)	24(65)	5(13)	37
Framycetin	11(24)	28(61)	7(15)	46
Povidone iodine	10(21)	30(64)	7(15)	47
Silver sulphadiazine	9(26)	20(57)	6(17)	35
Total	38(23)	102(62)	25(15)	165

Table 2. Bacterial isolates and their susceptibility to framycetin

Organism	Number of isolates	
	Total	Sensitive to Framycetin
E. coli	155	136(88%)
Staphylococci	240	223(93%)
Pseudomonas pyocyaneus	93	83(89%)
Klebsiella	36	27(97%)
Streptococcus	31	30(97%)
Proteus	27	22(82%)
Coliform	17	14(89%)
Total	599	535(89%)

Proteus and other coliforms in that order. The susceptibility of these isolates to Framycetin averaged 89% with a range of 82%–97%.

The correlation between swab culture and biopsy culture is given in (Tab. 3). The number of instances where both were positive was 280 and where both were negative 98. This constitutes a positive correlation of 70% (378 out of 537).

Table 3. Swab culture vs. biopsy. No. of instances

		Biopsy		
		+	−	Total
S	+	280(52%)	90(17%)	370
W	−	69(13%)	98(18%)	167
A				
B	Total	349	188	537(100%)

The two methods 'swab culture' and 'biopsy' do not differ significantly ($p > 0.10$ from McNemar's test).

The effect of topical antibacterial treatment on the area of wound at various time points and the percentage reduction thereof is given in (Tab. 4). All patients showed a progressive reduction in the area of wound with treatment, right from week 1. At the end of 4 weeks of therapy, Framycetin treated patients showed a reduction in area of 78.2% as against 56.1% in the Povidone iodine group and 61.8% in the Silver sulphadiazine group. These differences were significant in favour of Framycetin.

The progressive reduction of colony count on biopsy culture with treatment is given in (Tab. 5). Framycetin treatment produced a 92.1% reduction as early as week 1 as against

Table 4. Reduction in area of wound (cm^2) with treatment. Values represent mean (SE)

Treatment Group	Values					Percentage reduction			
	Pretreatment	Wk.1	Wk.2	Wk.3	Wk.4	Wk.1	Wk.2	Wk.3	Wk.4
Control	94.1 (12.8)	87.8 (11.8)	73.8 (9.7)	61.2 (7.2)	49.8 (4.4)	6.7 (1.3)	21.5 (2.6)	35.0 (3.1)	47.1 (2.6)
Framycetin	104.6 (14.6)	92.7 (12.7)	63.0 (7.3)	46.2 (5.8)	22.8 (3.1)	11.2 (1.9)	39.6 (3.4)	55.7 (4.2)	78.2 (6.9)
Povidone iodine	83.8 (11.8)	76.8 (10.1)	63.0 (7.5)	50.4 (5.3)	36.8 (3.8)	8.3 (1.4)	24.8 (2.9)	39.8 (3.0)	56.1 (4.9)
Silver sulphadiazine	102.7 (13.2)	93.8 (12.3)	74.3 (8.9)	59.9 (5.2)	39.2 (3.7)	8.7 (1.3)	27.6 (2.8)	41.7 (3.7)	61.8 (4.8)

Within Groups — All values significant from first week onwards (Paired t-test).

Between Groups— Framycetin superior to Povidone iodine and Silver sulphadiazine and all three better than control (Mann Whitney test).

Table 5. Reduction in colony count on biopsy culture (units of 10^6) with treatment. Value represent mean (SE)

Treatment Group	Values					Percentage reduction			
	Pretreatment	Wk.1	Wk.2	Wk.3	Wk.4	Wk.1	Wk.2	Wk.3	Wk.4
Control	4.72 (0.8)	3.76 (0.8)	2.87 (0.7)	11.50 (0.4)	0.40 (0.1)	20.3 (2.3)	39.1 (3.4)	68.2 (6.1)	91.5 (8.4)
Framycetin	5.11 (0.9)	0.40 (0.1)	0	0	0	92.1 (8.7)	100	100	100
Povidone iodine	4.80 (0.8)	2.44 (2.6)	2.14 (0.6)	0.48 (0.1)	0	49.2 (4.1)	55.6 (5.2)	90 (8.7)	100
Silver sulphadiazine	3.95 (0.7)	1.79 (0.4)	1.23 (0.3)	0.35 (0.1)		54.6 (4.9)	68.8 (6.3)	91.1 (8.9)	100

Within Groups — All values significant from first week onwards (Wilcoxon MPSR test).

Between Groups— Framycetin superior to Povidone iodine and Silver sulphadiazine and all three better than control (Mann Whitney test).

49.5% with Povidone iodine and 54.6% with Silver sulphadiazine. The control group had a reduction of 20.3%. These differences are significant in favour of Framycetin. Similar differences favourable to Framycetin were observed at weeks 2 and 3. By week 4, however, almost all cultures were negative in all groups.

A correlation of colony count on biopsy culture with clinical condition of the wound is given in (Tab. 6). Out of a total of 537 observations, 333 (62%) had colony counts of

Table 6. Correlation of colony count on biopsy culture with clinical condition of the wound

Wound	Colony count		Total
	$\geq 10^5$/g tissue	$< 10^5$/g tissue	
Unhealthy	333(62%)	11 (2%)	344
Healthy	16(3%)	177 (33%)	193
	349	188	537 (100%)

Colony count correlates with the appearance of wound ($p > 0.10$ from McNemar's test).

Table 7. Overall evaluation

Response	Number of patients (%)			
	Control	Framycetin	Povidone iodine	Silver sulphadiazine
Very good	7(19%)	19(41%)	4(9%)	4(11%)
Good	7(19%)	18(39%)	11(24%)	11(23%)
Fair	12(32%)	6(13%)	15(32%)	9(26%)
Poor	8(22%)	2(5%)	5(11%)	6(17%)
Very poor	3(8%)	1(2%)	12(24%)	5(14%)
Total	37(100%)	46(100%)	47(100%)	35(100%)

105 or more/g. of tissue and were clinically classifiable as unhealthy. 177 (33%) had colony count of 10^4 or less/g. of tissue and were clinically classifiable as healthy. As in the case of swab culture versus biopsy culture correlation, this picture points to a high degree of correlation between colony counts and clinical condition of the wound.

The overall evaluation of the response to treatment is presented in (Tab. 7). 80% of patients treated with Framycetin reported good to very good results as against 33% with Povidone iodine and 34% with Silver sulphadiazine.

Discussion

The mere presence of bacteria on the surface of the wound does not establish the diagnosis of wound infection. When a true wound infection does exist, the advancing front of infection is in the living tissues, not in the necrotic tissue or the purulent wound drainage (Zintel, 1956). Teptitz et al. (1964) showed that clinical features of burn sepsis appeared when bacteria crossed supraeschar and intraeschar areas and infected the subjacent viable tissue (Teptitz, 1964). Various workers observed in their studies that quantitative wound biopsy culture provides an accurate way of diagnosis of wound infection (Prueit, 1973; Hammer, 1975 and Bharadwaj, 1983).

This series has brought out the above phenomenon but has also indicated a possible cut-off point for quantitative bacteriological culture of wound biopsies below which even a positive surface culture loses its clinical significance. Only those patients with 10^5 or more bacterial colonies per gm. of wound tissue had clinically unhealthy wound surfaces. Contrary-wise, patients who had 10^4 or less bacterial colonies per gm. of wound tissue had clinically healthy wound surfaces. The point to be stressed here, is that unless a quantitative colony count is conducted on wound biopsies, the mere pre-

sence of organisms on surface swab culture may mislead the clinician with regard to the actual infective status of the wound.

It is significant to remember that a fair number of patients do show a discrepancy between swab culture and biopsy culture profiles, and given the direct correlation between the clinical status of the wound and the quantitative biopsy culture, it is advisable to rely on the latter when one is in doubt. Due practical consideration must however be given to the fact that the procedure of carrying out biopsy of a wound without anaesthesia cannot be recommended lightly as a routine bedside method for determining the infective status of the wound.

Staphylococci were the most frequently encountered pathogens in this study, although E. coli and Pseudomonas put together surpassed their number. If all Gram-positive and Gram-negative organisms are respectively added together, it is evident that an anti-microbial agent effective against Gram-negative bacteria as well as Gram-positive ones is called for in therapy. It may be mentioned here that chronologically speaking, Gram-positive organisms usually predominated during the first week, but the Gram-negative ones were more prevalent during the later stages. Similar observations were made by Bharadwaj et al. (1983).

Of the topical agents used, Framycetin was observed to be most effective both in vitro against the bacterial isolates and clinically as judged by reduction in wound area and colony counts on biopsies. The reduction in colony count obtained with Framycetin treatment also points to the penetration of the cream form into the dermis and subcutaneous tissues in effective bactericidal concentrations.

It is noteworthy that Framycetin was found to be superior to Povidone iodine and Silver sulphadiazine both in reducing colony counts and wound area. Out of the two, Silver sulphadiazine was marginally superior to Povidone iodine on both counts. Regarding Povidone iodine, it is clear that the bactericidal property of this preparation is governed by a highly complex set of factors that include free iodine generation, interaction with organic material, and so on (Gotterdi, 1983). One such variable can easily account for the apparent inadequacy of Povidone iodine in the control of infection in treated patients. With regard to Silver sulphadiazine, since it is known not to be so effective in the treatment of established infection, but only to reduce microbial colonies and the incidence of infection of burn wound (Mandell, 1985); the demonstrated superiority of Framycetin over this agent is easily explicable. It can thus be concluded that among the topical antimicrobial agents included in the present study, Framycetin sulphate is the most suitable for application over an open infected wound, whereas Silver sulphadiazine and Povidone iodine are less effective in treating established infections. Mere reliance on surface swab culture of wounds may often mislead the clinicians unless backed by quantitative wound biopsy cultures.

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