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Bacterial Colonisation of Lower Limb Ulcers and its Effect on the Success Rate of Skin Grafting - An Observational Study

AUTHOR

Abstract

Background: *Chronic leg ulcers are a significant cause of morbidity in developing countries like India, leading to excessive health care expenses and loss of effective work hours. Various novel methods are available, like split thickness skin grafting (STSG), vacuum-assisted closure, offloading technique nano silver dressings, etc. in order to facilitate earlier recovery with a varied response among the patients. Split thickness skin grafting is one of the main surgical procedures to repair soft tissue loss in patients with non-healing chronic wounds. Even when the prerequisites for successful grafting are met, the graft may fail because of bacterial infections in the site of ulcers. Hence in this present study, a quantitative bacteriological profile of granulating wound beds that were prepared for grafting was done pre-operatively to analyse the influence of bacterial bio-burden on the success rate of STSG. We also wanted determine the percentage of successful graft uptake on 10th post-operative period, assess the association of various bacterial growths with the successful graft uptake and also to determine other factors influencing the graft uptake.*

Methods: *This is a cross-sectional study was conducted from December 2018 to November 2019 among 100 individuals admitted in the Department of General Surgery in tertiary care hospitals with lower limb ulceration treated by skin grafting during a one-year period. Daily debridement and dressing of ulcers was done until healthy granulation tissue appeared. All patients were swabbed from their ulcers on admission, at 48 hours before skin grafting, and a third in the post-operative period during 5 to 10 days. The percentage of graft uptake in various bacterial groups was determined.*

Result: *In our study, a total of 100 patients who presented with an ulcer over lower limbs were taken into the study, and nearly two-thirds of the participants were females. Majority of the study participants were from the age group 51 to 80 years, followed by 31 - 50 years. The mean age was 47.71, with a standard deviation of 14.1 years. Most of the subjects stayed in the hospital for a period of 21 to 30 days, followed by 31 to 40 days. Diabetic ulcers formed the commonest (43.0%) type of ulcers, followed by traumatic (20.0%), venous (14.0%) and arterial (10.0%). Successful graft uptake was seen in the post-operative period in the 18 - 30 years age group (93.3 %) while it was admission stage in both 31 - 50 (90.2 %) and 51 - 80 (77.3 %) years age group subjects. There was no statistical significance. Regarding graft take up, it was more when there was no growth in swab result at admission. A similar result was observed during the pre-operative and post-operative periods. Graft take-up percentage was almost the same in both pre- and post-operative periods for staphylococcus (75.0 %), and mixed infections (50.0 %), Whereas the percentage of graft take-up, was more (80.0 %) during the post-operative period when the swab was tested positive for Enterobacteriaceae. Graft take up failure was more in mixed infections (50.0 %) followed by pseudomonas infection in both pre-operative and post-operative periods.*

Conclusion: *Findings of our present study concluded that the colonization of ulcers by Pseudomonas aeruginosa and Staphylococcus aureus relatively had poor graft survival, and the other bacteria like Enterobacteriaceae, etc., had a higher graft survival compared to other bacteria. This study thus emphasizes the importance of a quantitative and qualitative bacteriological culture of pre-grafted wound beds prior to skin grafting to determine the severity of infections in the wound bed, thereby preventing graft failure.*

Keywords: *Chronic Lower Limb Ulcers, Debridement and Dressing, Healthy Granulation Tissue, Skin Grafting*

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I. Background

An ulcer can be defined as a break in the epithelial continuity. In India, prevalence is between 1.5 to 3.0 per 1000 and it increases with age and is more than 2.0% with those aged over 80 years. Partial-thickness skin-grafting is a combination of operative and non-operative procedures directed at the underlying cause that can provide a role in the management of lower limb-ulceration. Skin grafting, especially split-thickness skin grafting, is one of the most widely used modalities in the treatment of ulcers. Most of the prerequisites for successful grafting known are adequate vascularized recipient bed, a good graft, accurate approximation, and

immobilization of the graft in relation to the ulcers in the focus of applying pop to the ulcers, avoiding the fluid–collection under the graft and best nursing care.

Even when these conditions are managed, the graft may fail because of bacterial infections in the site of ulcers. Six groups of bacteria have been noticed as reducing the success rate of skin-grafting - Beta-haemolytic streptococci, staphylococci, *E. coli*, pseudomonas, klebsiella and proteus. In developing countries like India, most of the patients are suffering from non-healing ulcers, and they come from poor-socio-economic status; that's why they cannot afford treatment and high-grade broad-spectrum antibiotics which suppress the above-mentioned bacterial action. A secondarily great deal of time and effort is spent in pre-operative and post-operative care to rid the ulcer of colonized bacteria. Anyway, there is small information on the effect of wound colonization on graft-uptake.

Aim

To analyse the bacterial colonization of lower limb ulcers and its effect on the success rate of skin-grafting.

Objectives

a) To determine the bacterial profile among the study subjects at various time periods.
b) To determine the percentage of successful graft uptake on 10th post-operative period.
c) To assess the association of various bacterial growths with the successful graft uptake.
d) To determine other factors influencing the graft uptake.

Figure 1. Traumatic Ulcer Over Foot

Figure 2. Healing of Ulcer After Graft

Figure 3. Diabetic Ulcer Over Leg

Figure 4. Healing of Ulcer After Graft

II. Methods

This is a cross sectional study conducted among one hundred consecutive patients who presented with lower limb ulceration and were admitted during December 2018 to November 2019 under the Department of General Surgery, SVRRGGH, Tirupati for split skin grafting.

Inclusion Criteria

1. Non healing ulcer on the lower limb.
2. Age more than 20 years of both gender.
3. Venous ulcer and arterial insufficiency ulcer over the lower limb.

Exclusion Criteria

1. Skin grafts in areas other than lower limbs.
2. Presence of the slough on the recipient ulcer area.

Sampling and Sample Size calculation

Every consecutive patient who presented to the outpatient department of General Surgery with lower limb ulcerations after considering inclusion and exclusion criteria were selected for the study.

Sample size calculation:

Considering prevalence of chronic leg ulcers (p) as 1.0% among adults as per the literature [x], q=100-p i.e., 99.0%, and with an absolute precision of 2% (d), z value being 1.96 at 95% confidence interval, the total sample size was estimated to be 95 based on the formula, $n=z^2(pq/d^2)$. The same was rounded off to 100. (Sharma R, Sharma RK, Goyal P, Sharma A, Suresh M, Saurav. Review of chronic leg ulcers over a period of one year in a tertiary care hospital in North India. Sch. J. App. Med. Sci., 2016; 4(4D):1392-7.)

After obtaining prior ethics approval for this study was obtained from the ethical committee of the institution and informed and written consent from all the participants, the study was undertaken.

Every patient was examined thoroughly, and blood was taken for investigations like haemoglobin, blood urea, serum creatine, serum electrolytes, and fasting blood sugar and post prandial blood sugar (PPBS). Their resting arterial ankle pressure was measured, and the venous system was assessed by Doppler ultrasound. Wherever appropriate, patients had venous surgery, arterial surgery, angioplasty, or lumbar sympathectomy before skin grafting.

The swabs from each patient were taken for pus culture and sensitivity at the time of admission and then at 48 hours before grafting (pre-operative period). Third swab was taken at the post-operative period on the 5^{th} and 10^{th} day. Ulcer size and graft uptake was assessed by tracing on graph paper.

Suitable patients underwent dressings, debridements, venous surgeries, and lumbar sympathectomy and amputations, etc., other treatments included a period of bed rest, limb elevation if there was no evidence of arterial disease, antibiotics were prescribed if there was evidence of cellulitis and surgical debridement was done if necessary before skin grafting. Graft taken from the thigh or any suitable region was fenestrated or meshed, and was then suitably positioned on freshly debrided ulcers in all cases. Appropriate sutures were put or were stapled, and also plaster of paris (POP) was applied to hold the graft in position. Cotton wool dressing was done, and the graft was inspected first on the 5^{th} post-operative day. Repeat grafts on all cells with rejected grafts were taken as new cases. After 5 days, the graft was inspected, if the graft was taken, the area was cleaned, redressed with saline, and a support bandage was applied. If skin graft take was doubtful and slough was present on the ulcer, the graft was rolled, cleaned with saline, and dressed. Graft uptake was assessed by the surgeon on day ten and at intervals after that.

Statistical Analysis

Data were recorded in MS Excel and SPSS version 18.0 software and Epitools online free version calculator was used for statistical analysis. The continuous variables were expressed in terms of mean and standard deviation and categorical data in terms of percentages/proportions. To assess the association between the factors influencing the successful graft uptake, Chi-square test or Fisher's exact tests were used appropriately. The difference in proportions was assessed using z-test for difference in proportions. A *P*-value of<0.05 was considered as statistically significant.

III. Results

In the present study, nearly two-thirds of the participants were females (70.0%), and the rest were males. Majority of the study participants were from the age group 31 - 80 years (85.0%). The mean age was 47.71, with a standard deviation of 14.1 years. Concerning the type of ulcer, majority (43. %) of the participants had diabetes, followed by traumatic (20.0%) and venous (14.0%), respectively. Only 10.0 % of the subjects had

arterial type of ulcer. Leg was the most commonly affected site in the lower limb (38.0%) and most i.e., 45.0% stayed in the hospital for 21 to 30 days period [Table-1]

The bacterial growth was positive among 86.0%, 63.0% and 69.0% at the time of admission, in the pre-operative period and at 10^{th} day of post-operative period respectively. Staphylococcus aureus was the commonest growth in all being (56.0%) at the time of admission, 36.0% in the pre-operative period and 27.0% in the post-operative period. [Table-2]

The proportion of successful uptake of the graft appeared to be higher among those with no bacterial growth on the 10^{th} post operative day (83.9%) followed by those who were positive for enterobacteriaceae (80.0%), staph. aureus (74.1%), pseudomonas (54.5%) and least with combined infection of staph. aureus and pseudomonas (50.0%). However, the proportions of successful uptake did not vary significantly among different bacterial growths when compared to pre-operative and post-operative period. It either did not vary among those with no growth also ($P>0.05$) [Table-3]

Though proportion of successful uptake of the graft at any time was lesser among those with bacterial growth, there was no significant difference among the bacterial growth during various periods of time with the successful uptake ($P>0.05$). [Table-4]

None of the factors like age-group, gender, pre-operative or the post-operative bacterial growths of staph. aureus, enterobacteriaceae, pseudomonas with or without staph. aureus were found to influence the successful uptake of the graft ($P>0.05$). [Table-5]

Variables	Frequency (n = 100)	Percentage (%)
Age Group in Years		
18 - 30	15	15.0
31 - 50	41	41.0
51 - 80	44	44.0
Gender		
Female	70	70.0
Male	30	30.0
Type of Ulcer		
Arterial	10	10.0
Venous	14	14.0
Mixed venous & arterial	13	13.0
Traumatic	20	20.0
Diabetic	43	43.0
Location of Ulcer		
Foot	30	30.0
Foot and leg	19	19.0
Leg	38	38.0
Thigh	9	9.0
Thigh and leg	4	4.0
Duration of hospital stay in days		
16-20	9	9.0
21-30	45	45.0
31-40	31	31.0
>40	15	15.0

Table 1. Distribution of the study participants based on socio-demographic details, location and type of ulcers and duration of hospital stay

Bacterial Swab Result	Frequency (n = 100)	Percentage (%)
At the time of admission		
No growth	14	14.0
Staph aureus	56	56.0
Pseudomonas	12	12.0
Enterobacteriaceae	10	10.0
Staph aureus+ Pseudomonas	8	8.0
Pre-operative period		
No growth	37	37.0
Enterobacteriaceae	15	15.0
Staph aureus	36	36.0
Pseudomonas	8	8.0
Staph aureus+ Pseudomonas	4	4.0
Post-operative period at 10^{th} day		
No growth	31	31.0
Enterobacteriaceae	25	25.0

Staph aureus	27	27.0
Pseudomonas	11	11.0
Staph aureus+ Pseudomonas	6	6.0

Table 2. Distribution of Study Participants by Bacterial Swab Results during different time periods

Growth status[§]	**Successful Graft uptake**				***P*-value**
	Total n for pre-operative period	**Pre-operative period**	**Total n for 10th post-operative period**	**On 10th post-operative day**	
No growth	37 (100.0)	32 (86.5)	31 (100.0)	26 (83.9)	0.38
Staph. aureus	36 (100.0)	27 (75.0)	27 (100.0)	20 (74.1)	0.47
Enterobacteriaceae	15 (100.0)	10 (66.7)	25 (100.0)	20 (80.0)	0.17
Pseudomonas	8 (100.0)	5 (62.5)	11 (100.0)	6 (54.5)	0.36
Staph aureus+ Pseudomonas	4 (100.0)	2 (50.0)	6 (100.0)	3 (50.0)	0.5

Table 3. Comparing the proportions of successful graft uptake during pre and the 10th post-operative period

[§]One-tailed z-test applied

Bacterial growth	**Successful Graft uptake at different time periods**		$\chi 2$ value (*P*-value)
	Yes	**No**	
At admission			
Yes	70 (85.4)	16 (88.9)	(1.00)[§]
No	12 (14.6)	02 (11.1)	
In the Pre-operative period			
Yes	44 (57.9)	19 (79.2)	3.54 (0.06)
No	32 (42.1)	05 (20.8)	
In the Post-operative period			
Yes	49 (65.3)	20 (80.0)	1.88 (0.17)
No	26 (34.7)	05 (20.0)	

Table 4. Association of bacterial growth with successful graft uptake in different time periods

[§]Fisher's exact test applied

Factors influencing the graft uptake	**Successful Graft uptake on 10th post-operative day**		$\chi 2$ value (*P*-value)
	Yes	**No**	
Age-group			
≤50	45 (80.3)	11 (19.7)	1.95 (0.16)
>50	30 (68.1)	14 (31.9)	
Gender			
Male	25 (83.3)	05 (16.7)	1.59 (0.21)
Female	50 (71.4)	20 (28.6)	
Swab result in pre-op period			
Staph aureus	27 (75.0)	09 (25.0)	1.28 (0.53)
Enterobacteriaceae	10 (66.7)	05 (33.3)	
Pseudomonas with or without Staph aureus	07 (58.3)	05 (41.7)	
Swab result on 10th post-op day			
Staph aureus	20 (74.1)	07 (25.9)	2.82 (0.24)
Enterobacteriaceae	20 (80.0)	05 (20.0)	
Pseudomonas with or without Staph aureus	09 (56.3)	07 (43.7)	

Table 5. Association of factors affecting the successful graft uptake on the 10th post-operative period

IV. Discussion

The study was conducted from December 2018 to November 2019; a total of 100 patients who presented with an ulcer over lower limbs were taken into study. In the present study, nearly two-third of the participants were females, and the rest were males. The purpose of this study was to know the bacterial burden on lower limb ulcers during admission, pre-operative, and post-operatively by pus culture and sensitivity and bacterial bio-burden, which influence the success rate of skin grafting. STSG is the standard gold treatment for skin defects due to chronic non-healing wounds. Even though the success rate of skin grafts depends on multiple factors, a well vascularised non-infected wound bed before grafting is crucial.

In our study, a total of 100 patients with an ulcer over lower limbs were considered, and nearly two-third of the participants was females, and the rest were males. Majority of the study participants were from the age group 51 - 80 years, followed by 31 - 50 years. The mean age was 47.71, with a standard deviation of 14.1 years. Regarding the number of days of hospital stay, most of the subjects stayed for 21 - 30 days, followed by 31 - 40 days. Only 9 % of the subjects had a stay of 16 - 20 days. Concerning the type of ulcer, majority (43.0

%) of the participants had diabetes, followed by traumatic (20.0 %) and venous (14.0 %), respectively. Only 10.0 % of the subjects had the arterial type of ulcer. About the gender distribution of type of ulcer, majority of the female participants had diabetic ulcers (40.0 %). A similar observation was seen in males (50.0 %). At the same time, no male participants had arterial ulcers. There was a statistically significant difference in proportions between gender and type of ulcer. Traumatic ulcers (33.3 %) and venous ulcers (26.7 %) were more common among the 18 - 30 years age group. At the same time, it was diabetic in subjects of the age group 31 - 50 years (46.3 % and 51 - 80 years (47.4 %).

In the present study, most of the participants had an ulcer on the leg (38.0 %) followed by the foot (30.0 %) followed by the thigh. In majority of the female subjects, the ulcer was located on the leg (35.7 %), followed by the foot (31.3 %). Similarly, most male subjects also had an ulcer on the leg (43.2%), followed by foot (26.7 %). While the location of ulcers among the subjects in the age group 18 - 30 years was more on foot (40.6 %), it was more on the leg in the subjects with age groups 31 - 50 years (41.5 %) and 51 - 80 years (36.4 %) respectively.

Out of the 86, more than half of the subjects (56.0 %) had *Staphylococcus aureus* in the swab, followed by Pseudomonas (12.0 %). Few subjects (8.0 %) had mixed infection. During the pre-operative period, majority (36.0 %) of the subjects had *Staphylococcus aureus* infection in the ulcer. About 37.0 % of the subjects had no growth in swab results. During post-operative period, over one quarter (27.0 %) of the subjects had *Staphylococcus aureus* infection, followed by Enterobacteriaceae (25.0 %). At the same time, nearly one-third of the swab results showed no growth of bacteria. During admission, majority of the female (53.0 %) and male (63.4 %) participants were tested positive for *Staph aureus.* In the pre-operative period, most female patients had no growth (38.5 %) in the swab test, while in males, 43.3 % were tested positive in a swab. During post-operative period, the swab test results showed that the share of Enterobacteriaceae was more in females (28.6 %). At the same time, it was *Staph aureus* in males (40.0 %). Swab test result at admission found that all the age groups had a significant proportion of *Staph aureus* infection (53.3 %, 73.2 % & 40.9 %, respectively).

Swab test of the pre-operative period showed that most of the 18 - 30 years and 51 - 80 years age group subjects had no growth (40.0 % & 34.1 % respectively). At the same time, it was a *Staph aureus* in 31 - 50 years age group people. However, there was no statistically significant difference in proportions.

Swab test of the post-operative period reported that there is an equal share of 33.3 % in no growth and Enterobacteriaceae infections in 18 - 30 years, whereas in 51 - 80 years age group subjects, most of them had *Staph aureus* infection (31.7 %). While in 51 - 80 years, majority had no growth (31.8 %), followed by Enterobacteriaceae (25.0 %). However, there was no statistically significant difference in proportions.

Regarding graft take up, it was more when there was no growth in swab result at admission. A similar result was observed during the pre-operative and post-operative periods. Further, in the pre-operative period, there was a statistically significant difference in proportions of graft take up according to bacterial growth in swab result. In both female (78.6 %) and male subjects (90.0 %), the graft was taken up successfully at admission.

Successful graft uptake was seen in post-operative period in the 18 - 30 years age group (93.3 %) while it was admission stage in both 31 - 50 (90.2 %) and 51 - 80 (77.3 %) years age group subjects. There was no statistical significance. Regarding graft take up, it was more when there was no growth in swab result at admission. A similar result was observed during the pre-operative and post-operative periods. Graft take-up percentage was almost the same in both pre- and post-operative periods for staphylococcus (75.0 %), and mixed infections (50.0 %), Whereas the percentage of graft take-up, was more (80.0 %) during the post-operative period when the swab was tested positive for Enterobacteriaceae. Graft take up failure was more in mixed infections (50.0 %) followed by pseudomonas infection in pre-operative and post-operative periods. Four-fifths of the participants did not have any complications. Fever (9.0 %) and pain (9.0 %) are complications in the remaining participants.

Few studies have analyzed the effect of bacteria on skin graft healing, particularly in the field of chronic lower limb ulceration. The number of organisms has been claimed to be critical, but results were not related to bacterial type. More recent analysis suggests that bacterial swab count taken from a single sample has minimal value as the range of values from a chronic ulcer can be very large. The type of bacteria present is more important. Beta-haemolytic streptococci and Pseudomonas were reported to cause skin graft failure in burns patients more than 30 years ago. Although a relationship between other bacteriological types and the failure of skin grafts has been postulated, this has never been demonstrated. Our study has confirmed that of Jackson et al. in that the isolation of Pseudomonas from an ulcer immediately before skin grafting significantly impairs skin graft take. The presence of Pseudomonas post-operatively also reduced skin graft healing, although a statistically significant difference was not found.

The finding that *S. aureus*, when present on an ulcer immediately before skin grafting, reduces graft take serves to confirm what was previously only suspected. Although its effect was not as large as that seen for Pseudomonas, it was sufficient to significantly reduce median graft take to 41 % and prolong the post-operative

stay of these patients. The finding that graft take, when *S. aureus* remains on the ulcer post-operatively, is further reduced to 74 %, suggests that *S. aureus* continues to impair graft healing and its eradication from a chronic ulcer is important.

Staphylococci secrete large number of toxins and enzymes, and it is the enzymes, which include hyaluronidase, fibrinolysis and proteases, that has been suggested to impair the ingrowth of capillaries through the fibrin layer that is laid down between the granulation tissue and the graft. It remains to be seen whether the concentration of staphylococci or its phage-type has a more significant effect on graft take.

The finding that *S. aureus* was present in 15 of the 16 ulcers that either failed to heal initially or that recurred later suggests a potential benefit from specific treatment aimed at eradicating this bacterium from an ulcer. The use of flucloxacillin orally is empirical; there is no evidence to support its use except, perhaps when cellulitis occurs. So far, the use of topical antibiotics has proved ineffective and a frequent cause of allergic reactions.

The study of traumatic ulcers has shown that in the absence of significant vascular disease, whether *S. aureus* is present or not, the immediate and long-term success of skin grafts is excellent. We have measured transcutaneous oxygen (TcPo2) levels in a subsequent group of patients with active ulceration and found that it can be shallow in the skin surrounding both venous and arterial ulcers. However, it is not reduced around traumatic ulcers. As oxygen is essential for wound healing, this low TcPo2 around vascular ulcers might explain our early and long-term failures.

The management of chronic lower limb ulceration is often empirical. The myth that an ulcer will almost universally recur after skin grafting can condemn the ulcer patient to a life-long rota of clinic attendance and district nurse visits. This study shows what can be achieved by an experienced team - a 90 % initial skin graft was taken with only seven ulcers still active after a median follow-up of 1 year. We accept that a proportion of these ulcers would have healed with outpatient treatment and, in some cases, pinch grafts. However, the 3 - 8 weeks of inpatient care is more than offset by the immediate relief of pain and the reduction in dressings and district nurse visits. The late failure rate of 8 % is disappointing and increases the follow-up period. We have found two groups unsuitable for a Thiersch graft-first, ulcers over lying bone or tendon, and second, patients with a fixed ankle and wasting of the calf muscle pump. Exclusion of these two groups, correction of underlying venous and arterial problems, treatment tailored to the swab results, and close follow-up could further improve the results of skin grafting in patients with chronic lower limb ulceration and deserves further study.

These observations were similar to the retrospective study by Gilliand EL et al. in which it was observed that analysis of the immediate pre-operative swab results suggested that it was Staphylococcus aureus and *Pseudomonas* which significantly affect skin graft healing, median graft take is reduced by 15 % ($P < 0.05$) and 40 % ($P < 0.01$) respectively when compared to the 'no growth' group. In another retrospective study, conducted by Høgsberg T et al. it was analyzed that that once the ulcers were infected with *Pseudomonas aeruginosa*, even weeks or months pre-operatively, the success of skin graft uptake was reduced. Their results showed that 33.3 % of ulcers with *Pseudomonas aeruginosa* were healed by the 12-week follow-up, while 73.1 % of ulcers without *Pseudomonas aeruginosa* were so by the same time.

The results of this present study were also similar to that of Jackson DM et al. in that the isolation of *Pseudomonas aeruginosa* from an ulcer immediately before skin grafting significantly impairs skin graft take. Unal S et al. had also observed that *Pseudomonas aeruginosa* was an equally prominent danger in skin graft survival.

Matsumura *et al*. showed that melting graft wound syndrome developed in 29 out of the 1359 patients evaluated. Swab wound cultures from these patients mainly grew *Staphylococcus aureus*, and none grew *Streptococcus* species. Jackson *et al*. showed that isolation of *Pseudomonas* from ulcers immediately before skin grafting significantly affected graft take. Other studies mention that wound *Pseudomonas aeruginosa* concentration greater than 105 colony-forming units/gm of tissue prevent wound healing. However, it has not been determined whether it is the number of bacteria or a toxin produced by these organisms that impedes the wound healing process.

Another study of *Pseudomonas* in chronic venous leg ulcers by Trine & Thomas showed that 33.3 % of ulcers with *P. aeruginosa* were healed by 12 weeks, while 73.1% of ulcers without *P. aeruginosa* were healed by 12 weeks. The P. *aeruginosa* in chronic venous leg ulcers has a considerable impact on partial take or rejection of spilt thickness skin graft. Gilliland had observed greater than 90 % uptake in 67 % of cases of ulcers colonized with gram-negative bacteria.

Wistrom et al. stated that chronic venous leg ulcers are contaminated or colonized with bacteria that seldom affect ulcer healing. According to their study, enterococci, anaerobic bacteria, and gram-negative bacteria, including *Pseudomonas* species, often colonize chronic ulcers but do not usually cause antibiotics requiring infection.

This differs from the study conducted by Høgsberg T et al. in which no statistically significant correlation could be detected between the clinical outcome and the other microorganisms. Also, in the study conducted by Gilliand EL et al. bacteria other than *Staphylococcus aureus* and *Pseudomonas aeruginosa* did not show any significant difference in the clinical outcome of graft uptake.

V. Conclusions

We have found two groups unsuitable for split-thickness skin graft - first, ulcer overlying bone or tendon, and second, patients with a fixed ankle and wasting of the calf muscle pump. Findings of our present study concluded that the colonization of ulcers by *Pseudomonas aeruginosa* and *Staphylococcus aureus* relatively low graft survival, and the other bacteria like Enterobacteriaceae, etc., had a higher graft survival compared to other bacteria. Exclusion of these two groups, correction of underlying venous and arterial problems, treatment tailored to the swab results, and close follow-up could further improve the results of skin grafting in patients with chronic lower limb ulcerations and deserves further study. The effect of bacteria on wound healing depends on the bacterial bio-burden in the wound and the virulence of bacteria. This study thus emphasizes the importance of a quantitative and qualitative bacteriological culture of pre-grafted wound beds before skin grafting to determine the severity of infections in the wound bed, thereby preventing graft failure.

Limitations of Our Study

A long term follows up of the patients to assess the graft uptake was not done due to non-feasibility. Anaerobic bacteria causing infection and hence graft failure were not studied because of a lack of resources.

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