Original Research Article

Bacterial colonization of leg ulcers and its effect on success rate of skin grafting

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ABSTRACT

Background: Leg ulceration is a common condition that affects many individuals. It is often treated by split skin graft. The aim of the study is to estimate the effect of bacterial colony on the skin graft and to evaluate the effects of diabetes and age on wound healing.

Methods: This is a prospective study of 50 patients who underwent SSG for leg ulcer in KIMS from June 2018 to May 2020. All patients underwent blood tests for diabetes and wound swabs were taken preoperatively and 5th post-operative day (POD). SSG take-up assessed on 8th POD.

Results: 62% of cultures were positive, of which Staph. aureus 48%, Klebsiella 6% Acinetobacter 4%, Citrobacter and E. Coli 2%. During the culture taken on 5th POD (1st dressing), Staphylococcus aureus was found in 42% of patients, and other bacteria put together 24%. Regarding take up, ulcers with Staphylococcus aureus had significantly reduced graft take up when compared to no growth, both during pre-op culture test and 5th POD dressings, by 24.18 and 33.165% respectively. The length of hospital stays for patients with positive Staphlycoccus aureus either in pre-operative or 5th POD swab was more by 15.25 compared to no growth.

Conclusions: For patients with Staphylococcus aureus on the ulcer, graft take up is less and post op stay is long, so, eradication of Staphylococcus aureus is essential. For patients with other bacteria, did not have significant difference with no growth group.

Keywords: Leg ulcer colonization, Staphylococcus aureus, Length of post-opertaive stay

INTRODUCTION

Leg ulceration is a common debilitating condition with a prevalence of 4.5/1000 population.[1] The incidence of acute wound was more than doubled at 10.5/1000 population.[2] Prevalence of ulceration increases with age and more than 2% in those over 80.[3] SSG is widely accepted method for coverage of open wounds.[4] Fibrin is the agent responsible for adherence of skin graft to the wounds. Different organisms cause grafting to fail by production of proteolytic enzymes which dissolve the important fibrin scaffold which in-turn causes failure of applied skin grafts.[5] Beta haemolytic streptocci and *Pseudomonas* are known to reduce the success rate of skin grafting.[6,7] Factors like age and diabetes are known to affect wound healing which are being evaluated.

Objectives of the study

To find the incidence of various types of bacteria in pre-grafted ulcers, to estimate the grafts take rate in leg ulcers with different bacteria and compare their graft take rates with leg ulcers having no growth, to estimate the length of post operative stay in patients having leg ulcers with different bacteria and compare with patients having leg

ulcers with no growth and to determine the effects of associated factors like age and diabetic status on graft take.

METHODS

The study is conducted on patients with leg ulcers in between 18-80.

Study design: Prospective observational study.

Study area

Karpaga Vinayaga Institute of Medical Sciences and Research Centre, Chinna Kolambakkam, Madhuranthagam taluk.

Study period

June 2019 to May 2020.

Inclusion criteria

Traumatic ulcers on the legs, patients having raw area on the legs after wound debridement in patients with infective conditions like necrotizing fasciitis etc, diabetic patients with leg ulcers and patients having raw area on the legs secondary to burns.

Exclusion criteria

Patients with ulcers having slough, unhealthy granulation tissue and pus discharge, patients with ulcers due to arterial insufficiency, venous and vasculitis ulcers, patients with ulcers having pus culture swab results turned positive for *Streptococci* and *Pseudomonas*, patients with comorbidities like chronic kidney disease, coronary artery disease and cerebrovascular accidents were excluded from the study, patients with ulcer floor formed by bone, ligaments and cartilage and patients with skin diseases causing ulcers on the leg.

Method of collection of data

All patients fulfilling inclusion and exclusion criteria, belonging to age 18-80 years were included in this study after taking informed consent. Patients and relatives were explained about the split skin grafting procedure and the study, necessary approval was obtained from them prior to intervention. Data regarding the name, age, sex, education, occupation address, chief complaint, treatment history before admission for present complaint, history regarding the mode of onset of disease, past medical and surgical history, personal history was collected from patient and accompanying relatives. Intra operative and post-operative status of leg ulcer and graft were noted systematically.

Sample size

The sample size was 50.

Sampling method

Random sampling.

Method of clinical survey

50 patients who were satisfying both inclusion and exclusion criteria having ulceration on the leg were taken into the study. In most of the cases the ulcer size was greater than 5cm^2.

Figure 1: Ulcer before grafting.

All patients were examined fully, haemoglobin and random blood sugar levels were checked.

Necessary blood transfusions were given for anaemic patients and skin grafting was done only after the haemoglobin was brought to the normal range.

Anti-diabetic treatment was given for diabetics, skin grafting was done only after control of blood glucose levels.

All patients with low serum protein levels were given high protein diet and necessary albumin transfusions; skin grafting was done only after correcting hypoproteinemia.

In infective conditions, thorough wound debridment was done and corresponding antibiotics were administered; skin grafting was done only after the ulcer developed good healthy red granulation tissue with minimal exudates.

In all patients, skin grafting was done after ulcer developed healthy red granulation tissue with no pus discharge and no slough.

The graft was taken from thigh, fenestrated and placed on an ulcer with healthy red granulation tissue in one or more pieces to achieve maximal coverage.

Swabs were taken 48 hours before split thickness skin grafting and also at the time of 1st post operative dressing i.e 5th post operative day. Swabs were cultured and bacterial isolates were identified using standard techniques.

Figure 2: Ulcer after split thickness graft.

Cuticell was applied over skin graft and pressure dressing was applied on the recipient site with roller gauze. On the donor site, vaseline gauze was applied and pressure dressing was applied with roller gauze.

After 5 days, the graft was inspected. If the graft had taken and the area was clean, it was redressed with cuticell and a support bandage with roller gauze was put for a further 3 days. Where the skin graft take was doubtful and there was slough on the ulcer, the graft was rolled, cleaned with saline, and dressed with cuticell soaked in Betadine on a daily basis. Graft take was assessed on day 8 and at 3 days intervals thereafter. In most of the patients strict bed rest was maintained for 8 days. Skin grafted site is considered to be healed if the graft take up is >95%. This skin graft take up assessment will be done on 8th post skin grafting day and looked whether graft has taken up. Donor site dressing was removed and checked on 10th post skin grafting day to look for any signs of infection and also whether donor site site is healing or not. All patients were discharged from the hospital only when the skin graft appeared stable and after full mobility has been regained.

Follow up

All patients were followed up for a maximum period of 12 weeks.

RESULTS

This study deals with studying the frequency distribution of presence of bacteria 48 hours before SSG and also during first post-operative dressing. The frequency distribution of etiology-burns, infection and trauma are also studied. One-way ANOVA is used to test if there is any significant difference in mean 'initial graft take (%)' among no growth, *Staphylococcus aureus* and other bacteria for both 48 hours before SSG and also during first post-op dressing. In all the analyses, significance level is taken to be 0.05 (i.e., if the p≤0.05, we reject the null hypothesis or we say that the hypothesis is statistically significant) and the tests are two-tailed. Statistical analysis was carried out using statistical package, SPSS (version 22.0.0.0).

Here the frequency distribution of sex is studied. It can be observed that out of the 50 patients, 34 (68%) of them are males and 16 (32%) of them are females. The frequency distribution table is given below.

Table 1: Frequency distribution of sex.

	Frequency	%	Cumulative percentage
Females	16	32.0	32.0
Males	34	68.0	100.0
Total	50	100.0	

The frequency distribution of etiology- burns, infection and trauma are given below.

Table 2: Frequency distribution of etiology.

	Frequency	%	Cumulative percent
Buns	9	18.0	18.0
Infection	23	46.0	64.0
Trauma	18	36.0	100.0
Total	50	100.0	

Table 3: Frequency distribution of different bacteria on leg ulcers.

	Frequency	%	Cumulative percent
No growth	19	38	38
S. aureus	24	48	86
Klebsiella	3	6	92
Acinetobacter	2	4	96
Citrobacter	1	2	98
E. coli	1	2	100
Total	50	100	

Statistical analysis was done for mean initial graft take among patients with no growth, *S.aureus* and other bacteria. The results showed that for culture swabs taken 48 hours before SSG, patients leg ulcers with *S.aureus* has significantly reduced mean graft take by 24.189% when compared to no growth and leg ulcers with Other bacteria had no significant difference in mean graft take when compared to no growth.

At the time of 1st postoperative dressing, the results showed a similar relationship to graft take up. Leg ulcers with *S.aureus* has significantly reduced mean graft take by 33.165% when compared to no growth; other bacteria had no significant difference in mean graft take when compared to no growth.

Length of stay

The mean length of post-operative stay is compared for patients with swabs taken at 48 hours before SSG and first post-operative dressing. The results are given below.

Table 4: Length of stay among patients with different bacteria before SSG and on 1st postop dressing.

	Length of stay	Bacteria before SSG	Bacteria post-op dressing
No growth	Mean	13.789	12.706
	SD	5.534	4.058
S. aureus	Mean	29.042	33.000
	SD	12.842	10.173
Other bacteria	Mean	20.571	16.167
	SD	5.127	4.629

It can be observed that for both 48 hours before SSG and first post-op dressing, mean length of post op stay is highest for patients with *S.aureus* , followed by other bacteria and lowest for no growth.

Statistical analysis was done for evaluating length of postop stay among patients with leg ulcers having *S.aureus* , other bacteria and no growth. It was found that mean length of post operative stay for patients having leg ulcers with *S.aureus* during 48 hours before SSG is significantly more by 15.25 days when compared to patients with leg ulcers having no growth.

There is no significant difference in mean length of post operative stay when compared between the patients having leg ulcers with other bacteria and no growth during 48 hours before SSG.

At the time of 1st post operative dressing also the mean length of post operative stay for patients with leg ulcers with *S.aureus* is significantly more by 20.294 days when compared to patients with leg ulcers having no growth and also mean length of post operative stay for patients with leg ulcers with *S.aureus* is significantly more by 16.83 days when compared to patients with leg ulcers having other bacteria.

Here the initial graft take is compared among the two different age groups 0-50 years and >50 years. The descriptive statistics is given below.

Table 5: Comparison of initial graft take among age groups.

Age (in years)	N	M	SD	Std. mean
0-50	28	86.786	12.781	2.415
>50	22	69.091	20.681	4.409

From the Table 6, it can be observed that the mean initial graft take is lower for >50 years than 0-50 years of age. Independent sample t-test is used to test the null hypothesis that there is no significant difference in mean initial graft take among the age groups.

Table 6: Independent sample t-test among different age groups.

t	df	Sig. (2-tailed)	Mean Difference	Std. error difference
3.718	48	0.001	17.695	4.759

From the above table it can be concluded that there is significant difference in mean initial graft take (t (48)=3.718, p=0.001) among the age groups. However the patients were not matched for diabetic status, etiology of ulcer and type of bacteria present.

Comparison of mean 'initial graft take' among diabetic status irrespective of colonizing bacteria

Here the initial graft take is compared among the diabetic status of the patients. The descriptive statistics is given below.

Table 7: Descriptive statistics for graft take between diabetic and non-diabetic.

Diabetes Mellitus	N	M	SD	Std. error mean
Absent	25	82.400	18.771	3.754
Present	25	75.600	18.502	3.700

From the above table it can be observed that the mean initial graft intake is lower for people with diabetes.

Table 8: Independent sample t test.

t	df	Sig.(2-tailed)	Mean difference	Std. error difference
1.290	48	0.203	6.800	5.271

Independent sample t-test is used to test the null hypothesis that there is no significant difference in mean initial graft take among diabetic and non-diabetic patients.

From the above table it can be concluded that there is no significant difference in mean initial graft take (t (48)=1.290, p=0.203) among diabetic and non- diabetic patients. However the patients were not matched for age at time of grafting and type of bacteria present.

DISCUSSION

This study is conducted to evaluate the incidence of various bacteria in ulcers before grafting and also to determine the effect of different types of bacteria on the graft at 48 hours before split skin grafting and on fifth post op day. This study evaluated the uptake of graft, length of

postoperative stay and also to demonstrate the effect of factors like age, diabetic status on graft take.

In our study out of fifty patients, 9 (80%) patients were due to burns, 23 (46%) patients were due to infective etiology. 18 (36%) patients were due to traumatic etiology. Most ulcers were infected and needed wound debriment.

In our study, the incidence of different bacteria in preoperative ulcers was 62%. Of which *Staph. aureus* 48%, *Klebsiella* 6% *Acinetobacter* 4%, *Citrobacter* and *E. Coli* 2%. The rest 38% did not show any growth.

Kontiainen et al have shown that pathogens isolated from lesions in skin and soft tissue were *S.aureus* and streptococcus amounting to 50%, *Pseudomonas* and other bacteria ampunting to 10% of lesions.[8]

Halbert et al found out that common isolates were *S.aureus* 48%, mixed coliforms 28%, *Pseudomonas* aeroginosa 21%, and anaerobes 17%.[9]

Greenwood et al reported that most common pathogens in burns wound were *S. aureus* 75%, *P. aeroginosa* 25%, streptococcus 20%, and various coliform bacilli 5%.[10]

In our present study, when compared to above mentioned study absence of *Streptococci* and *Pseudomonas*. Leg ulcers with streptococcus and *Pseudomonas* were not included in the present study because skin grafting on leg ulcers with those bacteria is contraindicated.

Hogsberg et al in a retrospective study in patients with chronic venous leg ulcers *Pseudomonas aeroginosa* was found in most of the patients and had considerable impact on partial uptake and rejection of SSG.[11]

Wilson et al in a study conducted on patients with burns requiring split skin graft suggested that non-group A *Streptococci* maybe more pathogenic than previously recognized in this particular aspect.[12]

In the Gjodsbol et al study proved that beta haemolytic *Streptococci* and *Pseudomonas* were reported to cause graft failure.[13]

The above mentioned studies proved that beta haemolytic *streptococci* and pseudomonas infections of leg ulcers are contraindications for skin grafting because of skin graft failure. Hence leg ulcers with beta haemolytic *Streptococci* and *Pseudomonas* were not included in the study.

The number of organisms has been claimed to be critical, but results were not related to bacterial type.[14,15] A study done by Schneider et al suggests that a bacterial swab count taken from a sample has very limited value as the range of values from a chronic ulceration be very large.[16] The type of bacteria is important. Quantitative Assessment of bacterial invasion of chronic ulcers by Schneider et al shows that bacterial count made on a single tissue sample

from a chronic ulcer has only limited value as guide to the surgeon in determining the optimal time to perform a surgical closure.[17]

Gilliland et al demonstrated that presence of bacteria in initial swab test did not affect the outcome of SSG.[18] Presence of bacteria in the immediate preop period (48 hours before SSG) and postop period (POD5) affected the SSG take. According to our study mean SSG take for patients with S. aureus 48 hours before SSG had significantly (24%) reduced take compared to no growth group. For swabs taken at POD5 the mean uptake of graft reduction for *S. aureus* group is 33.16% compared to the no growth group. The above results suggest that leg ulcers with *S.aureus* significantly reduces graft take.

Staphylococci secrete hyaluronidase, fibrinolysins and proteases which are known to impair the ingrowth of capillaries through the fibrin layer that is laid down between the granulation tissue and the graft which is suggesting us the reason for reduced graft take in wounds colonized with *Staph aureus*.[5]

Patients having leg ulcers with other bacteria in culture swabs taken at both 48 hours before STSG and at 1st post-operative dressing had no significant difference in mean graft take when compared to no growth.

Wistrom et al stated that enterococci, other coliforms and anaerobic bacteria do not usually cause antibiotic requiring severe infection and hence may not affect graft take significantly.[19]

Bacterial colonization in leg ulcers also affected the length of postoperative stay. In our present study, it is observed that mean length of post operative stay for patients with leg ulcers having *S.aureus* in culture swabs taken at 48 hours before SSG is significantly more by 15.25 days when compared to patients with leg ulcers having no growth.

Mean length of postoperative stay for patients with leg ulcers having *S.aureus* at 1st post-op dressing (POD-5) is significantly more by 20.294 days when compared to patients with leg ulcers having no growth and also mean length of postoperative stay for patients with leg ulcers having *S. aureus* at 1st post-op dressing is significantly more by 16.83 days when compared to patients with leg ulcers having other bacteria.

Our present study results suggest that presence of *S.aureus* delays the wound healing and increases the length of post operative stay.

Madsen et al did a study on 59 venous ulcers to evaluate the possible influence of selected bacterial species on healing of leg ulcers.[20] In their study they followed up the patients for 180 days. Ulcers with growth of *S.aureus* healed significantly more slowly than those without when relative areas were compared on day 180 (p=0.0079).

Complete healing within the observation period of 180 days was observed in 21.6% of patients with S. aureus and 62.5% of those without (p=0.0278). The results also suggest a healing delay caused by *S. aureus*. According to the present study, the results suggest that *S.aureus* significantly reduces the graft take and also prolongs the length of postoperative stay by delaying wound healing. So, eradication of *S.aureus* from the wound is essential.

In present study out of 50 patients the youngest was 24 years and the oldest were 80 years. The graft take was compared among the patients less than 50 years age group and greater than 50 years age group. The results suggested that there is significant difference in mean initial graft take among the age groups. There was a significantly higher incidence of graft take in the patients aged less than 50 years than those aged 50 years and above. However, the patients were not matched for diabetic status, etiology of ulcer and type of bacteria present.

In present study, 25 patients were diabetics and 25 patients were non diabetics. The graft take was compared among diabetics and nondiabetics. The results suggested that that the mean initial graft intake is lower for people with diabetis having 75.6% and non-diabetics having 82.4% but there was no statistically significant difference However, the patients were not matched for age at time of grafting and type of bacteria present.

Ramanujam et al showed that diabetic patients without comorbidities had no significant difference in healing times compared to non-diabetic patients for STSG.[21]

In our present study all 50 patients were discharged by 45 days. Out of 50 patients chosen for the study except 2 patients all of the rest patients leg ulcers are healed by the time of discharge. Those two patients had their leg ulcers with *S.aureus* both during 48 hours before SSG and at the time of 1st postoperative dressing. Those two patients had small proportion of unhealed wound and were discharged with anti-staphylococcal antibiotics. All the patients were followed up for 3 months. In the present study the 12-week outcome can be interpreted as a predictor for the long-term outcome. Kirsner et al reported similar findings of a critical period of 3 months postgrafting after which, if the graft was intact, it tended to remain so.[22] By the end of 3 month follow-up, all 50 patients legs were found to be healed.

Limitations of the study

Although the research was carefully prepared, I am still aware of its limitations and short comings. The study was conducted only on small size of patients who came to our hospital, however to generalized the results for larger groups, the study should have involved more participants. The time from origin of ulcer to time of skin grafting was also not determined. While evaluating the graft take among age groups, the patients were not matched for diabetic status, etiology of ulcer and type of bacteria present. While comparing the graft uptake among the diabetic status, the patients were not matched for age, etiology of ulcer and type of bacteria present.

Follow up was limited to only the period of hospital stay with no long term follow up.

CONCLUSION

There was high incidence of bacterial colonisationin leg ulcers in Karpaga Vinayaga medical college, Madhuranthagam. Present study showed that presence of infection both during 48 hours before SSG and also at the time of 1stpost operative dressing reduced the graft uptake. However, the type of bacteria was also important.

Skin graft uptake was significantly less when leg ulcers had *S.aureus* both during 48 hours before SSG and also at the time of 1stpost operative dressing. Leg ulcers with *S.aureus* had delayed wound healing and increased length of post operative stay. So, eradication of *S.aureus* from the wound is essential. Leg ulcers with other bacteria did not have significant difference in graft uptake when compared to no growth hence not a contraindication to skin grafting. Graft uptake was not significantly affected by diabetic status of the patient.

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