IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 18, Issue 9 Ser.12 (September. 2019), PP 05-08
www.iosrjournals.org

Bacteriological Analysis of Skin Graft Infections in a Tertiary Care Teaching Hospital, Coimbatore

S.Rishi Kumar[1], Dr.N.Bharathi Santhose [2]
[1](Final year MBBS Student, Coimbatore Medical, College)
[2](Senior Assistant Professor, Department of Microbiology, Coimbatore Medical College)
Corresponding Author: Dr.N.Bharathi Santhose

Abstract : *Bacterial infections are a major cause of delayed wound healing and is the common complication leading to failure of skin graft .The objective of this study was to analyze the bacterial infections in skin grafting in relation to time and their antibiotic resistance patterns. The study was conducted on 50 patients who were undergone skin grafting at a plastic surgery department in a tertiary care teaching hospital for 3 months duration. A total 250 swabs were collected, 5 from each patients. The isolates were identified by standard microbiological techniques and antimicrobial susceptibility testing was done by Kirby Bauer's Disc Diffusion Method. Detection of Extended-spectrum beta-lactamase (ESβL), Metallo-β-lactamase(MβL) and Amp C-type β-lactamase was done as per CLSI guidelines. The tissue defects were grouped according to the cause as follows: traumatic tissue defects(41.1%), burns(28.3%), vascular ulcers(10.2%), and flap donor-site defects(20.4%). All the samples collected at the time of admission and after 1 week of skin graft showed no growth. Pseudomonas aeruginosa was the organism most commonly isolated followed by Proteus spp., after 2 week of skin grafting. Other organisms isolated were Klebsiella spp., Escherichai coli, Staphylococcus and Enterococcus spp. Graft loss secondary to infection was observed in 11 patients (22%). Most of the isolates were Multidrug resistant. Among the gram negative bacterial isolates, 57% were ESβL producers, 12% were AmpC producers and 12% were MβL producers. Pseudomonas aeruginosa was the predominant ESβL and MβL producer. The gram negative bacteria are more predominant cause of skin graft infections than the gram positive bacteria. Hence, the importance of proper pre & post-operative wound care by aseptic dressing, strict adherence to infection control measures, prevailing & emerging drug resistance pattern in a hospital setting must be educated and implemented to prevent skin graft failure because of infections.*

Keywords: *skin grafting, bacterial infection, antibiotic resistance, P.aeruginosa,, β-lactamases*

Date of Submission: 09-09-2019 Date of Acceptance: 25-09-2019

I. Introduction

The human skin is a vital organ with several major functions: protection, sensation, thermoregulation, excretion, absorption, metabolism and even non-verbal communication.[1] Any breach in skin integrity due to injuries, burns etc., may lead to the disruption of one or more functions as well as pain, discomfort and possible bacterial infection. In order to restore the functions of the damaged skin, skin graft surgeries are being performed with the intention of excelerate healing, minimize scarring and to provide a physical barrier to the exposed host bed against infection **.**

The two main types of Skin-Graft surgeries are Full Thickness Graft and Split-thickness graft (SSG) which is classified as : 1. Thin SSG , 2. Intermediate SSG and 3. Thick SSG. The Prerequisites for successful skin graft are good graft, adequately vascularized recipient bed, accurate approximation and immobilization of the graft in relation to the ulcer, avoiding fluid collections beneath the graft, and good nursing care. Even though these prerequisites are meticulously taken , the graft may fail due to bacterial infection.[2] The success of any organ transplantation is associated with two cross linked & inter dependent clinical events ; Rejection and Infection.[3] However, the incidence of infection affects the outcome of the transplant surgery especially skin grafting.

The surface of the chronic wound is likely to host commensal flora, and it is more likely that an in-depth residing bacterium is more pathogenic than a superficial one.[4] Bacterial load, virulence, host immune response, age of patient, extent of injury, depth of wound are the risk factors influencing skin graft infection. The bacteria mainly responsible for this type of infection are Staphylococcus spp., Klebsiella spp., Pseudomonas spp., Streptococcus spp., and sometimes anaerobic bacteria like Bacteroides etc., are also reported. Many of these bacteria's are acquired from the hospital settings, that are resistant to antibiotics to varying degrees. Viral & Fungal infections are very rare, in case of skin graft surgeries. Therefore,

bacteriological culture of wounds before and after skin grafting should be performed to understand the burden of infection related graft loss.[5]

Hence, this study is undertaken to analyze the bacterial infections in skin grafting and their antibiotic resistance patterns, which would definitely guide the treating surgeon to choose the appropriate antibiotics and also help them to improve infection control measures against such infection, thereby reducing overall infection related graft rejections and ensuring better clinical outcome in future.

II. Objectives

1. To isolate & identify the predominant causative bacteria responsible for the skin graft infections with respect to different time interval (i.e., pre & post operatively) among the study population.
2. To study any antibiotic resistance patterns like ESβL, AmpC β-lactamases, MβL producers among the Gram negative bacterial isolates.

III. Material & Methods

This Prospective Hospital-based study was conducted in the Department of Microbiology, Coimbatore Medical College and Hospital over a period of 3 months. A total of 250 swabs collected from 50 patients from the Plastic Surgery Department who satisfied the inclusion criteria as posted for either full- or split- thickness skin grafts to reconstruct soft-tissue defects were included in the study. The demographic information of the cases like age, sex, risk factors and dates of sample collection were recorded. The study was conducted after obtaining the Institutional Human Ethical Committee Clearance and getting informed consent from the patients included under the study.

The first sample was collected at the time of admission from clinically deep area of wound prior to any cleansing i.e., on Day 0 and subsequent samples are collected after surgical excision and grafting in specified time intervals on week 1, week 2, week 3 & week 4. All specimens were inoculated on 5% Sheep blood agar, Mac Conkey agar and Chocolate agar plates and incubated for 24 - 48 hrs at 37 deg C . The anaerobic culture was performed manually using Anaerobic jar with Gas pack. The bacterial isolates were identified by standard microbiological techniques.[6] Antibiotic susceptibility testing were done on Muller-Hinton agar by Kirby-Bauer's Disc Diffusion Method according to CLSI-2018 guidelines.[7] The gram negative isolates were subjected to screening for the production of ESBL by double disk approximation test. The organisms which were screened and found positive for ESBL were subjected to confirmatory test by phenotypic confirmatory test. AmpC disk test was also done for the Meropenem resistant strains for detection of AmpC β-lactamases. All Carbapenem-resistant isolates were subjected to detection of Metallo β-lactamase (MBL) production by Modified Hodge's test and EDTA disk synergy test. Methicilin Resistance for Staphylococcus sp were detected by disc diffusion method using 30µg cefoxitin disc. The diameter of the zone around the disc was measured and the results were interpreted.

IV. Results

About fifty patients who received either full- or split-thickness skin grafts to reconstruct soft-tissue and skin defects admitted in Plastic surgery department, CMCH were included in the study. Among the study population 39 were male patients and 11 were female patients. The average age was 40 years ranging from 20 to 70 years. About 42% of the samples (n=21) were collected from raw area over the left leg and about 16% of the samples(n=8) were collected from right leg. The least number of samples were collected from left ankle and left thigh (about 2% samples).

The tissue defects were grouped according to the cause as follows: traumatic tissue defects(41.1%), burns(28.3%), vascular ulcers(10.2%), and flap donor-site defects(20.4%). Graft loss secondary to infection was observed in 11 patients (22%) especially in cases of chronic vascular ulcers in lower extremities.

Among the 50 samples collected at 2 week after the graft surgery, 35 samples (70%) were positive for the presence of infection and 15 samples (30%) were negative for the presence of infection. No obligate anaerobic bacteria were isolated in this study. Among the aerobic bacteria, Pseudomonas aeruginosa was the commonest gram negative bacilli isolated followed by Proteus spp. No organisms were isolated on before the graft (Day 0) and after the graft (first week). Gram negative bacteria's were more prevalent among the samples collected on second week after the graft which gradually reduced on subsequent weeks. Mixed cultures were not seen in the study. Staphylococcus spp. and Enterococcus spp. was isolated only in four samples & in two samples collected on second week after the graft surgery respectively. On the other hand single isolation of Pseudomonas aeruginosa 24% on week 2, and 18% on week 3, 8% on week 4 after the graft surgery. Proteus spp. was isolated in 16% of the samples on week 2, 12% of the samples on week 3, and 6% of the samples on week 4 after the graft surgery. Escherichia coli was grown in 10% of the samples on week 2, 6% of the samples on week 3, 2% of the samples on week 4 after the skin graft surgery. Klebsiella spp. was isolated in 6% of the samples on week 2, 2% of the samples on week 3 postoperatively. Staphylococcus spp. was grown

in 8% of the samples on week 2, 2% of the samples on week 3, whereas Enterococcus spp. growth was isolated in 4% of the sample on week 2 after the skin grafting. The various aerobic bacteria isolated from sequential culture of the skin graft was depicted in the (Table 1). The distribution of various bacterial isolates among the indication for skin graft was shown in the (Table 2). Among the gram negative bacterial isolates, 35% were extended spectrum beta lactamases producers,18% were AmpC β-lactamases producers and 7% were MβL producers (Table 3). Pseudomonas aeruginosa was the predominant ESβL and MβL producer. No MRSA & MSSA were observed in this study.

V. Tables

TABLE 1: Various aerobic bacteria isolated from sequential culture of the skin graft

Bacteria isolated	WEEK 1	WEEK 2	WEEK 3	WEEK 4
Number of Culture	50	50	50	50
Culture Positive	Nil	35	20	08
Culture Negative	50	15	30	42
Pseudomons aeruginosa	Nil	12	09	04
Proteus mirabilis	Nil	08	06	03
Escherichia coli	Nil	05	03	01
Klebsiella pneumoniae	Nil	03	01	Nil
Staphylococcus sp	Nil	04	01	Nil
Enterococcus sp	Nil	02	Nil	Nil

TABLE 2: Distribution of various bacterial isolates among the indication for skin graft

Bacteria isolated	Total	Traumatic Tissue defects	Burns	Vascular ulcers	Others
Pseudomonas aeruginosa	25	14	5	4	2
Proteus mirabilis	17	6	8	3	-
Eschericia coli	09	5	2	1	1
Klebsiella pneumoniae	04	2	2	-	-
Staphylococcus sp	05	1	3	1	-
Enterococcus sp	02	-	2	-	-

TABLE 3: Distribution of ß -lactamases producers among different GNB isolates

	Pseudomonas aeruginosa	Proteus mirabilis	Escherichia coli	Klebsiella pneumoniae
ESBL	8	6	3	2
Amp C	4	3	2	1
MBL	3	0	0	1

VI. Discussion

Skin graft are more prone for variety of complications leading to graft loss. A wide range of factors are believed to adversely influence skin graft take up; hematoma or shearing movements, inadequate compliance, deficient blood supply, presence of micro thrombi in dermal blood vessels, local fibrin deficiency in wound and infection.[8] In our study, bacteria was not isolated before skin graft and all the patients were on antibiotic treatment and proper dressing on alternate days. There was no sign of any infection till the end of first week of grafting. After 14th day of grafting, the most common infection that occurred in skin graft patients was by Pseudomonas aeruginosa. Analysis of the results obtained after 14th day of the graft surgery showed that 70% of the patients were positive for growth. This is in concordance with result of the study conducted by Loren G. Miller in which it was found that 60% patients who were treated in the ambulatory setting were categorized as abscesses or cellulitis. [9] Out of the 35 samples positive for the growth after 14th day of the graft, it was found that 71% of the samples were found to be positive for Pseudomonas spp. This was followed by Proteus spp. which was isolated in 49% of the samples and Escherichia coli which was isolated in 26% of the samples. This was in contrast to the results of the study conducted by Sakir Unal in which Pseudomonas was isolated in only 58.1% of the samples followed by Staphylococcus aureus, Enterobacter, Enterococci and Acinetobacter. [10] Infection may be due to acquired from hospital or may be due to poor nursing care, unhygienic practice followed by dressing. 30% of the samples showed no growth probably due to antibiotic therapy. The results of the study also showed that 65% of the gram negative isolates were resistant to two or more antibiotics i.e., mutlidrug resistant strains particularly ESβL, MβL & Amp C *β-lactamases* producers which is in concordance with the study by Vinod Kumar CS etal.[11]

Skin graft loss due to infection accounted for only minor part in the literature with very few publications on deterioration of skin grafts due to P. aeruginosa particularly in the chronic lower limb ulcerations.[12] Sakir Unal et al found that P. aeruginosa was an equally prominent danger as Streptococcus pyogenes in skin graft survival in routing plastic surgery practice.[10] McGregor in contrast claims that infection with P. aeruginosa reduces graft up take by 5-10%[12]. It has been reported that even with effective treatment of P. aeruginosa, the results observed was deterioration of skin grafts because of either insufficient eradication

from recipient wound bed or persistence of P. aeruginosa by their ability to colonize and proliferate in the form of biofilms. The clinical implications of bacterial biofilms are particularly noticeable in case of any chronic infections.[13]

Pseudomonas aeruginosa is well recognized for forming chronic biofilm-based infections in their hosts. Infection with P. aeruginosa prior to surgery, reduces graft take significantly. This indicates that P.aeruginosa resides deep down in the tissue, and is protected from antibiotics and also imprevious to immune defense mechanism due to biofilm formation. [14] It had been explained in various studies that P. aeruginosa like organisms with multi drug resistance and inherent nature of biofilm production may contribute to non healing of wounds resulting in chronic ulcers, thereby success rate of skin grafting will be deteriorated even in the presence of aggressive treatment and meticulous infection control measures. In our study, the rate of bacterial isolation from the skin graft after week 3 to week 4 had been decreased and incidence of delayed wound healing among the infected cases was not observed.

VII. Conclusion

This study showed that majority of the post operative samples during the week 2 were found to be infected with bacteria with resistant to multiple antibiotics, which can potentially result in graft loss. However, we observed skin graft failure related to infection only in 11 patients(22%) . In condition like persistence of deep seated colonization, aggregation as biofilm and hidden infection, it is impossible to detect by routine bacterial culture using superficial wound surface swab sampling . Hence, various sampling technique in addition to direct swabs like by collecting 3 to 4 representative tissue samples at the wound site including tissue biopsy from the margins of the skin graft and biofilm assays may be done to detect the hidden bacteria causing infections. As biofilm producing bacteria's are always associated with antibiotic failure and infection are resolved only on surgical debridement, use of biofilm inhibitor products would be an effective and ideal remedial strategy along with antibiotics and anti- inflammatory agents , which are currently under clinical trials. Bacteria might also be transmitted from one patient to another by inadequate sterilization of instruments or could be introduced into clean wound through faulty dressing technique.

The study had few limitations like that would have been more effective if larger number of sample size were analyzed, follow-up of few patients could not be completed due to short duration of hospital stay of those patients and role of biofilm production among the isolates from skin graft loss patients was not attempted. Hence, it is highly recommended to expand further by studying the specific profile of graft-bacterial infection , time-related changes among the dominant colonized bacteria with host flora and any propensity of biofilm production which will definitely helpful to reduce the overall infection related to graft loss.

In addition to this, proper wound care by aseptic dressing, strict adherence to infection control measures, knowledge on prevailing & emerging drug resistance pattern in a hospital settings, choosing the specific & appropriate prophylactic antibiotics will always play a crucial role in reducing the burden of these infection, thereby failure of skin graft will be prevented.

Acknowledgements

The authors would like to thank the all the faculty of Department of Plastic Surgery for their continuous support in patient selection and guidance throughout this study.

References

[1]. Timmons J :Skin function and wound healing physiology. Wound Essentials 1:8-17(2006)
[2]. E Leslie Gilliland. Bacterial colonisation of leg ulcers and its effect on the success rate of skin grafting. Anne R Coll Surg Engl 1988:(70):105-8.
[3]. Brunicardi.F.C., Anderson.D.K., Billar.T.R., Dunn.D.L., Hunter.J.G., Matthews.J.B., and Pollock.R.E., Ed.Schwartz's Principles of Surgery, 9th edn. McGraw-Hill Education.
[4]. Tine Yding Wolff, Thomas Bjarnsholt, Klaus Kirketerp-Møller and Trine Rolighed Thomsen. Influence of Microorganisms on the Healing of Skin Grafts from Chronic Venous Leg Wounds.Skin Grafts - Indications, Appl Curr Res 2011:277-90.
[5]. S Geethabanu and R Vanaja. A Study to Analyse the Influence of Bacterial Bio-Burden on the Success Rate of Split Thickness Skin Grafting. J Clin Diagn Res 2018;12(6):DC23-DC6.
[6]. Collee JG, Marr W. Culture of bacteria. Mackie and McCartney Practical medical microbiology. 14th edn. Edinburg: Churchill Livingstone.1996:95-149.
[7]. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 28th ed. CLSI document M100-S 28. Wayne, PA. (2018)
[8]. Høgsberg T, Bjarnsholt T, Thomsen JS, Kirketerp-Møller K. Success Rate of Split-Thickness Skin Grafting of Chronic Venous Leg Ulcers Depends on the Presence of Pseudomonas aeruginosa: A Retrospective Study. 2011;6(5):1-6.
[9]. Long G. Miller; et al: Incidence of skin and soft tissue infections in ambulatory and impatient setiings. BMC Infectious Diseases, Volume 15(362); July 2015.
[10]. Sakir Unal; et al: Analysis of skin-graft loss due to to infection: infection-related graft loss, Volume 55(1); July 2005.
[11]. Vinod kumar C.S, Shubha V.Hegde, Mayuri K.S, Nitin Bandekar, Chetan Kumar S and Basavararajappa K.G:Analysis of skin graft for bacterial infection in a tertiary health care centre.International Journal of Pharma and Bioscience 2015 July: 6(3):(B) 1256-1263.
[12]. McGregor AD, McGregor IA Fundamnetal Techniques of Plastic Surgery. 10th:35-59,(2000).
[13]. Davies D. Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov, 2:114-22, (2003).
[14]. Boutli-Kasapidou F, Delli F, Avgoustinaki N, Lambrou N, Tsatsos M, et al. What are biofilms? Evaluation and management in open skin wounds. J Eur Acad Dermatol Venereol, 20:743-5, (2006).