

IDENTIFICATION OF FUNGAL PATHOGENS IN BURNS PATIENTS WITH REFERENCE TO CANDIDA

Kandati Jithendra¹, Rama Mohan Pathapati², Premanadham Nadakuduru³, Madhavulu Buchineni⁴, Srinivas Pasupuleti⁵, Sreeram Sateesh⁶

HOW TO CITE THIS ARTICLE:

Kandati Jithendra, Rama Mohan Pathapati, Premanadham Nadakuduru, Madhavulu Buchineni, Srinivas Pasupuleti, Sreeram Sateesh. "Identification of Fungal Pathogens in Burns Patients with reference to Candida". Journal of Evidence based Medicine and Healthcare; Volume 2, Issue 1, January 05, 2015; Page: 1-8.

ABSTRACT: BACKGROUND: Infection with microbes in burns patients is a leading cause of morbidity and mortality. The present study was aimed to study the fungal pathogens from infected burns patients periodically during their stay in the hospital from wound surface, blood and intravascular devices and to identify and differentiate candida species. **METHODS:** This hospital based study was conducted during June 2011 to May 2013. Patients with greater than 25 - 30% burns of total body surface area (TBSA) and hospitalized in burns unit for a minimal duration of hospitalization of 7 days were included in the study. Specimens such as wound swabs, blood and intra vascular devices were collected at the end of 1st, 2nd, 3rd and 4th week, processing, isolation, identification and antibiogram of the isolates were done as per standard procedures. **RESULTS:** To analyze the systemic fungal infection in burn patient's Seven hundred and eight (708) blood cultures were collected and processed at regular intervals with isolation of 54 (7.6%) pathogens. Findings clearly indicates C.albicans as the most common pathogen 32 (59.26%) isolated followed by non-albicans group. In non-albicans group C.tropicalis was the most common followed by C.parapsoliasis and rest is C. krusei, C. kefir, C. glabrata and C. guilliermondii. Intravascular devices also act as sources for the systemic invasion of pathogens, Eight hundred and eighty four (884) IVD's were processed with isolation of 42(4.8%) pathogens. We found that C.albicans was the most common isolate 71.43% (30) followed by non-albicans group. In non-albicans group C.tropicalis (6) was the common followed in the order of C. parapsoliasis (4), C.krusei (1) and C. guilliermondii. **CONCLUSION:** We highlighted the incidence of fungal wound infection is increasing due to wide spectrum antibiotic administration. Candida albicans was the common pathogen isolated from all the specimens followed by C.tropicalis and C.parapsoliasis. Next common fungus isolated was Aspergillus spp which was further confirmed by histopathological correlation. Patients with fungal wound infection had long duration of hospital stay and systemic invasion had more mortality. Continuous surveillance of microorganisms and their antibiotic resistance can improve the efficacy of infection control programs in a burn unit.

KEYWORDS: Fungi, Candida, burns, Drug sensitivity, Time dependent emergence of pathogens.

INTRODUCTION: Burns are considered as a serious and devastating form of trauma which causes serious mortality and morbidity. Infections in burns patients with complications like sepsis, inhalational injury account for 75% of deaths in burns patients even though advances in surgical and medical management like early wound closure, tangential skin excision and infection control practices have been introduced.^(1,2,3) The breached skin in the burn wound is the hallmark of

ORIGINAL ARTICLE

thermal injury. Three distinct zones have been identified in the burn wound which includes i) Zone of coagulation ii) Zone of stasis and iii) Zone of hyperemia.^(4, 5) The subsequent infection of the burn wound followed by systemic infection is directly proportional to the extent of burn surface area.⁽⁶⁾ Associated with the destruction of the skin, depression of hosts cellular and humoral immune systems are key factors which promote the infection of wound and sepsis in these patients.

The site of thermal injury is sterile initially later on followed by colonization and invasion of pathogens. The type and the number of microorganisms which colonize the burn wound influence the future risk of invasive wound infection and sepsis.⁽⁷⁾ Within 48 hours gram positive bacteria located in the deep sweat glands and hair follicles colonize and invade the wound later on followed by gram negative bacteria, further yeasts which are derived from hosts normal intestinal and genito urinary tracts and also from the hands of health care workers.^(8,9) Because of presence of large number of virulence factors and emergence of antibiotic resistance, gram negative pathogens became dominant pathogens in burn wound infections and sepsis. Colonization of the burn wounds with yeasts and fungi occur late after second week due to long duration of broad spectrum antibiotic administration and associated compromised immune status of the host.^(10,11) However due to wide spread broad spectrum antibiotic administration and increased hospital infection control practices bacterial wound infections have declined. But the incidence of fungal wound infections (FWI) has not declined.⁽¹²⁾ However previous studies have demonstrated the association between TBSA and occurrence of fungal super infection.⁽¹²⁾ Burn wound infections due to fungi like *Candida* spp, *Aspergillus* spp, are common but opportunistic fungi like *Alternaria* spp, *Fusarium* spp, *Rhizopus* spp and *Mucor* spp are right now emerging as important fungal pathogens in burns patients. Fungal infections in burns are more common after second week of injury. The source of *Candida* spp is mostly endogenous i.e. from gastro intestinal or respiratory tract of the patient and infection appears as darkening of wound. Infections with *Rhizopus* spp, *Mucor* spp although rare are able to cause more mortality because of their ability to spread across the fascial planes and can invade the vasculature.⁽¹³⁾ Many of the studies clearly indicates *Candida* as the most common fungal pathogen in both local and systemic infections. The present study was aimed to study the fungal pathogens from infected burns patients periodically during their stay in the hospital from wound surface, blood and intravascular devices and to identify and differentiate *Candida* species. This study would enable to set up a separate burn management protocol in the hospital which helps to start empirical local and systemic antifungal therapy before the results of microbial culture become available and limit the septic episodes in the burn patients.

MATERIALS & METHODS: A hospital based study was conducted at Narayana medical college and Hospital, which is a tertiary care medical hospital, Nellore, Andhra Pradesh, India from June 2011 to May 2013.

INCLUSION CRITERIA: Patients who attended the surgical emergency of the hospital immediately after burns and admitted in to the Burns unit with a minimal stay period of 7days

ORIGINAL ARTICLE

and burn wound covering > 25 - 30% of Total body surface area (TBSA) were included in the study.

EXCLUSION CRITERIA: Patients who had burn injury with partial skin thickness burns <25% of TBSA, Full skin thickness burns <5% were treated as outpatients. The patients who were admitted after 72hrs of burn injury were also not included in the study.

DETAILS OF THE STUDY: In the present study, three hundred and fifty four patients were enrolled as per the inclusion criteria, in which 188 were males and 166 females. On admission routine investigations has been performed and documented along with variables like sex, age, type of burns and TBSA (Total body surface area). The specimens like swab, blood and IVD's from the patients were subjected to routine culture tests at regular intervals.

For isolation of pathogens, Swabs (Sterile cotton tipped) from the surface of wound and deeper parts were collected after cleaning with sterile normal saline and 70% alcohol periodically starting from the day of admission extending to the date of discharge or death. In case of a dry wound the swab was moistened with normal saline. Few of the swabs were collected during surgical debridement and prior to grafting. These swabs were transported and processed immediately at the Central Microbiology Laboratory, plated on Sabourad's Dextrose agar, Hi-chrome Candida differential agar [Hi media lab, Mumbai] incubated at 37°C aerobically for 24-48 hrs.

The blood samples from the patients were collected aseptically at the end of 1st, 2nd, 3rd and 4th week to demonstrate the time pattern of pathogens, irrespective of the presence or absence of signs and symptoms of Sepsis. The samples were processed by Automated BACTEC 3D blood culture system.

The tips of the removed IVD's were collected and processed for isolation of pathogens at regular intervals, by plating on Sabourad's dextrose agar, Hichrome Candida differential agar [Himedia lab, Mumbai] incubated at 37°C aerobically for 24-48 hrs.

The Growth on SDA was observed within 24- 48hrs and identified as Candida by gram staining. Other fungi like Aspergillus, Fusarium, Rhizopus, Mucor was identified by colony morphology and Lacto phenol cotton blue mount (LPCB). Punch biopsy was taken from the wound suspected with non-Candida fungal infection and confirmed by pathological examination by GMS (Gomori methanamine silver) staining. Candida albicans was identified by performing germ tube test and non -albicans group was inoculated on Candida differential agar and incubated for 24-48 hrs. Color of the colonies was noted and species identified based upon manufacturer's instructions and various literatures. Antifungal susceptibility testing of the fungus was not performed due to technical difficulties.

RESULTS: The study group included Three hundred and fifty four patients with 188 (53.2%) males and 166 (46.8%) females admitted in Burns unit of the Narayana medical college and Hospital, and fulfilled the inclusion criteria. The data clearly indicates that the male female ratio as 1:0.9, mean age of the patients as 24 ± 1.5 years (Range 1-82years) and mean TBSA percentage as 15% (Range 28-88%). (Table-1) The most common cause of burn injury was

ORIGINAL ARTICLE

Flame burns 262 (74%) followed by scalds 58(16.4%), electrical 24(6.8%) and other types 10(2.8%). (Table-1)

A total of 1062 wound swabs were collected at regular intervals from the burns patients. Findings indicate that 902 (84.93%) of the swabs exhibited no fungal growth and 160(15.07%) showed fungal growth. The minimal positivity was observed during the 1st week of injury (2-5%) and started to increase from second week (12.5%) thereafter with maximum by end of 4th week. (35.8%) (Table-2). Our study clearly indicates *C. albicans* was the predominant isolate from the wound swabs 100(62.5%), followed by Non-*albicans* group 46 (28.75%), *Aspergillus* spp 8 (5%), *Rhizopus* spp 4(2.5%) and *Fusarium* spp 2(1.25%). Among the Non-*albicans* group were *C. tropicalis* 12, *C.parapsoliasis* 10, *C. guillermondii* 8, *C. krusei* 6, *C.kefyr* and *C. glabrata* 5.

To analyse the systemic fungal infection in burn patient's Seven hundred and eight (708) blood cultures were collected and processed at regular intervals with isolation of 54 (7.6%) pathogens. Findings clearly indicates *C. albicans* as the most common pathogen 32 (59.26%) isolated followed by non-*albicans* group. In non-*albicans* group *C.tropicalis* was the most common followed by *C. parapsoliasis* and rest are *C.krusei*, *C.kefyr*, *C.glabrata* and *C.guillermondii*. (Table-4)

To asses that intravascular devices act as sources for the systemic invasion of pathogens, Eight hundred and eighty four (884) IVD's were processed with isolation of 42(4.8%) pathogens. We found that *C. albicans* was the most common isolate 71.43% (30) followed by non-*albicans* group. In non-*albicans* group *C. tropicalis* (6) was the common followed in the order of *C. parapsoliasis* (4), *C. krusei* (1) and *C. guillermondii* (1) [Table-4].

DISCUSSION: Burn wound provides excellent nourishment for colonization of fungi, the incidences being around 20%.⁽¹⁴⁾ Susceptibility of the burns patients to fungal infections is associated with three important factors which are disruption of dermal integrity, depressed immune function and prolonged antibiotic administration. Our present study was aimed to isolate and identify the fungal pathogens from the wound swabs, blood and IVD's at regular intervals. Many studies report that fungal infection in burns patient's increases with duration starting from second week of injury. In our study the maximum isolation was seen during the fourth week starting from the second week. Regular periodic collection of wound swabs indicated that *C.albicans* was the most common fungal pathogen throughout the study. The findings of our study correlates with many studies performed earlier.⁽¹⁵⁻¹⁷⁾ Our present study also clearly indicates that non-*albicans* groups are emerging fungal pathogens in burns patients. *C. tropicalis* in the group was most common in all the specimens followed by *C. parapsoliasis*. However the distributions of the pathogens are dependent upon the environment in the hospital and variable from place to place. The finding of our study concurs with the findings of Mathews M S et al who indicates *C. tropicalis* as emerging pathogen in burns patients.⁽¹⁸⁾ A study by Gaoxing Luo et al suggests that wound culture from an infected patient should be supported with specimens like blood, urine, Central lines and sputum etc.⁽¹⁹⁾ Identification of the genus (and often species) causing FWI has become important for patient care, as no one agent can provide adequate empirical therapy in all infected patients. Our study clearly indicated that *Aspergillus* spp, *rhizopus* spp, *Mucor* spp are rare but emerging pathogens and depends upon many environmental and

ORIGINAL ARTICLE

host factors. In depth analysis of the study totally indicates *C. albicans* as the most common pathogen followed by non-*albicans* group with *C. tropicalis* being most common in all the specimens processed. Growth of *Aspergillus* spp on sabourads dextrose agar was correlated with histopathological examination from punch biopsy of the wound and staining by GMS. Our study clearly indicated that histopathological examination of the wound is as reliable as swab cultures in invasive fungal infection of the wound.

Invasive candida infections in burns patients has become a common cause of mortality and morbidity with the prevalence of colonization from 13 to 31.8 %.⁽¹⁹⁾ Many studies conducted earlier mentions *C.albicans* as fourth common pathogen which cause septicemia in burns patients. The risk factors associated with invasive fungal infection includes greater TBSA, burn associated hyperglycemia, usage of central venous catheters, advanced patient age, prior antibiotic coverage and long duration of hospital stay.⁽²⁰⁾ Our study clearly demonstrates that *C. albicans* as a major pathogen with 59.26% and non-*albicans* group forming 40.74% which also suggests a rise in the non *albicans* group due to emergence of resistance to *C. albicans*. Among the non-*albicans* group *C. tropicalis* was predominant followed by *C. parapsoliasis*. Studies conducted by Alexandra et al, Pedrosa A F et al and Naveen Kumar et al from India suggests *C. albicans* as the most common followed by *C. tropicalis* which are similar to our findings in the study.^(15,20,21)

Few studies conducted earlier reported central venous lines as possible source of pathogens in sepsis. We investigated by culturing the tips of Intra vascular devices. Data of our study shows *C.albicans* as the major isolate from the devices followed by *C. tropicalis* and *C. parapsoliasis*. There was a similar pattern of isolation from blood culture and IVD's indicating that central lines may be colonized by the fungi with dissemination from the source leading to Candidemia.

CONCLUSION: In our study the incidence of fungal wound infection is increasing due to wide spectrum antibiotic administration. *Candida albicans* was the common pathogen isolated from all the specimens followed by *C. tropicalis* and *C. parapsoliasis*. Next common fungus isolated was *Aspergillus* spp which was further confirmed by histopathological correlation. Patients with fungal wound infection had long duration of hospital stay and systemic invasion had more mortality. In view of the above, serious strategies need to be employed to prevent the development of FWI. Avoidance of potted plants, flowers, effective air filtration facilities need to be employed in the burn units. Emergence of azole resistance in *albicans* is alarming threat; hence inappropriate systemic or topical application prophylactically may be curtailed to stop this.

REFERENCES:

1. Atiyeh, B. S., S. W. Gunn, and S. N. Hayek. 2005. State of the art in burn treatment. *World J. Surg.* 29: 131–148.
2. Baker, C. C., C. L. Miller, and D. D. Trunkey. 1979. Predicting fatal sepsis in burn patients. *J. Trauma* 19: 641–648.
3. Bang, R. L., P. N. Sharma, S. C. Sanyal, and I. Al Najjadah. 2002. Septicaemia after burn injury: a comparative study. *Burns* 28: 746–751.

ORIGINAL ARTICLE

4. Gibran, N. S., and D. M. Heimbach. 2000. Current status of burn wound pathophysiology. *Clin. Plast. Surg.* 27: 11–22.
5. Heideman, M., and A. Bengtsson. 1992. The immunologic response to thermal injury. *World J. Surg.* 16: 53–56.
6. Santaniello, J. M., F. A. Luchette, T. J. Esposito, H. Gunawan, R. L. Reed, K. A. Davis, and R. L. Gamelli. 2004. Ten year experience of burn, trauma, and combined burn/trauma injuries comparing outcomes. *J. Trauma* 57: 696–700.
7. Erol, S., U. Altoparlak, M. N. Akcay, F. Celebi, and M. Parlak. 2004. Changes of microbial flora and wound colonization in burned patients. *Burns* 30: 357–361.
8. Manson, W. L., H. J. Klasen, E. W. Sauer, and A. Olieman. 1992. Selective intestinal decontamination for prevention of wound colonization in severely burned patients: a retrospective analysis. *Burns* 18: 98–102.
9. Manson, W. L., P. C. Pernot, V. Fidler, E. W. Sauer, and H. J. Klasen. 1992. Colonization of burns and the duration of hospital stay of severely burned patients. *J. Hosp. Infect.* 22: 55–63.
10. Burdge, J. J., F. Rea, and L. Ayers. 1988. Noncandidal, fungal infections of the burn wound. *J. Burn Care Rehabil.* 9: 599–601.
11. Desai, M. H., and D. N. Herndon. 1988. Eradication of *Candida* burn wound septicemia in massively burned patients. *J. Trauma* 28: 140–145.
12. Becker WK, Cioffi WG Jr, McManus AT, et al. Fungal burn wound infection. A 10-year experience. *Arch Surg.* 1991; 126: 44–48.
13. Church Deirdre, Elsayed Sameer, Reid Owen, Brent Winston, and Robert Lindsay (2006): Burn Wound Infections. *Clin Microbiol Rev.* 19 (2): 403–434.
14. Jefferson Lessa Saores de Macedo, Joao Barberino Santos (2005): Bacterial and Fungal colonisation of Burn wounds. *Mem Inst Oswaldo Cruz. Rio de Janeiro* 100 (5): 535–539
15. Navin Kumar Goyal · Madhuri A. Gore · R. S. Goyal (2010) Fungal colonisation in burn wounds: An Indian scenario. *Indian J Surg (January–February 2010)* 72: 49–52.
16. Chakrabarti A, Nayak N, Kumar PS, et al. (1992): Surveillance of nosocomial fungal infection in burn care unit. *Infection* 20: 132–135.
17. Mousa HA (2003): Anaerobic, aerobic and fungal burn wound infections. *J Hosp Infect* 37: 317–323.
18. Mathews MS, Samuel PR, Suresh M (2001): Emergence of *Candida tropicalis* as the major cause of fungaemia in India. *Mycoses* 44: 278–280.
19. Macedo JL, Santos JB. Bacterial and fungal colonization of burn wounds. *Braz J Infect Dis* 2005; 100: 535-9.
20. Pedrosa AF, Rodrigues AG. Candidemia in burn patients: figures and facts. *J Trauma* 2011; 70: 498–506.
21. Alexandra Fochtmann A, Christina Forstnerb, Michael Hagmann et al. Predisposing factors for candidemia in patients with major burns. *Burns* 2014 (press article).

ORIGINAL ARTICLE

Sex	Male	188 (53.2%)
	Female	166 (46.8%)
Type of burns	Flame	262 (74%)
	Scalds	58 (16.4%)
	Chemical	24 (6.8%)
	Others	10(2.8%)

TABLE 1: Variables among burns patients (n=354)

TIME & No OF SPECIMENS RESULT	3RD DAY	7TH DAY	10TH DAY	14TH DAY	21ST DAY	28TH DAY	TOTAL
	n =188	n= 165	n =194	n= 192	n =144	n=179	1062
Sterile	184 (98%)	157 (95%)	176 (90.7%)	168 (87.5%)	102 (70.8)	115 (64.2%)	902 (84.93%)
Positive	4(2%)	8 (5%)	18 (9.3%)	24 (12.5%)	42 (29.25)	64 (35.8%)	160 (15.07%)

TABLE 2: Percentage Of Fungal Cultures Isolated From Burn Wounds

SPECIMEN &No RESULTS	BLOOD CULTURE	IVD'S
		n = 708
No fungal growth	654 (92.4%)	842(95.2%)
Fungal growth	54 (7.6%)	42 (4.8%)

TABLE 3: Percentage Of Fungal Cultures Isolated From Blood & Ivd's

NAME OF FUNGI	WOUND SWAB	BLOOD CULTURE	IVD
	NUMBER (%)	NUMBER (%)	NUMBER (%)
C. albicans	100 (62.5)	32 (59.26)	30 (71.43)
Non-albicans group	46 (28.75)	22 (40.74)	12 (28.57)
Aspergillus sp	8 (5%)	NI	NI
Fusarium sp	2 (1.25%)	NI	NI
Rhizopus sp	4 (2.5)	NI	NI

TABLE 4: Fungal pathogens isolated

ORIGINAL ARTICLE

	Wound swab	Blood culture	IVD
C. tropicalis	12	7	6
C. kefyr	5	2	NI
C. krusei	6	2	1
C. glabrata	5	2	NI
C. parapsilosis	10	6	4
C. gullermondii	8	3	1

TABLE 5: Non-Albicans Group

AUTHORS:

1. Kandati Jithendra
2. Rama Mohan Pathapati
3. Premanadham Nadakuduru
4. Madhavulu Buchineni
5. Srinivas Pasupuleti
6. Sreeram Sateesh

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Microbiology, Narayana Medical College & Hospital, Nellore, A. P.
2. Associate Professor, Department of Pharmacology, Narayana Medical College & Hospital, Nellore, A. P.
3. Assistant Professor, Department of Microbiology, Narayana Medical College & Hospital, Nellore, A. P.
4. Associate Professor, Department of Pharmacology, Narayana Medical College & Hospital, Nellore, A. P.

5. Research Scientist ARC, Department of Microbiology, Narayana Medical College & Hospital, Nellore, A. P.
6. Professor, Department of General Surgery, Narayana Medical College & Hospital, Nellore, A. P.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Rama Mohan Pathapati,
Associate Professor Pharmacology,
Narayana Medical College,
Nellore, A. P.
E-mail: pill4ill@yahoo.co.in

Date of Submission: 19/12/2014.
Date of Peer Review: 20/12/2014.
Date of Acceptance: 29/12/2014.
Date of Publishing: 31/12/2014.