Fungal colonisation in burn wounds: An Indian scenerio

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Abstract Burn patients are susceptible hosts for fungal colonisation.

Aim To study incidence and profile of fungal colonisation of burn wounds.

Materials and methods This prospective study was conducted in 201 consecutive adult patients (39 male and 162 female) with burn wounds, admitted from October 2005 to September 2006. Wounds with clinical suspicion of fungal colonisation were biopsied and examined by wet smear, Potassium hydroxide (KOH) mount, nigrosin stain, 1% Acid fast bacilli (AFB) stain, Gomori methenamine silver (GMS) stain, histopathological examination and fungal culture. All wounds with suspicion of fungal colonisation were treated with topical application of miconazole ointment mixed with povidone-iodine/silver sulphadiazine topically and itraconazole (oral) systemically.

Results Fungal infection of burn wound was suspected in 77 patients (38.31%) with 20–70% total body surface area (TBSA) burns. Ninety-two biopsy samples were collected from 77 patients. On investigations, fungal colonisation was confirmed in 35 patients. Culture was positive in 23 patients with *Candida* in 12 and *Aspergillus* in 4. GMS stain and KOH stain showed sensitivity and specificity of 70.8% and 86.5%, respectively for detecting fungi in burn wound. Nigrosin stain was negative in all patients. Duration

of hospitalisation was more in patients having fungal colonisation without any significant effect on the mortality

Discussion In 38.31% of patients fungal colonisation was clinically suspected and it was confirmed in 17.41% of 201 patients. GMS stain and KOH stain were observed to have acceptable sensitivity and specificity. With increasing prevalence of *Aspergillus*, fluconazole cannot be relied upon in controlling fungal colonisation and drugs like itraconazole may have to be used as empirical therapy.

Conclusion Fungal colonisation of burn wound is not uncommon and should be suspected. Fungi detected most commonly were *Candida* and *Aspergillus*. KOH and GMS stain were identified as reliable, simple and inexpensive methods for confirming fungal colonisation.

Keywords Burn · Fungal colonisation · *Candida* · *Aspergillus*

Introduction

Burn injury is a major health problem in India. Infection is the leading cause of morbidity and mortality in burns and remains one of the most challenging concerns in the management of burnt patient. Fungal wound colonisation with the possibility of invasive infection is a probability in burn patients, in view of use of antibiotics for prolonged periods and immunosuppression associated with burn injury. Clinical research pertaining to fungal infection has largely been a neglected area in India and this is an attempt to study current fungal profile in burn wounds and to suggest guidelines based on this study.

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Materials and methods

This prospective study was carried out in burn patients admitted from October 2005 to September 2006 to the burn unit at Lokmanya Tilak Municipal (LTM) General Hospital, Mumbai, India. The aim of the study was to determine incidence of fungal colonisation of burn wound and to identify the types of fungi.

Povidone iodine was the topical antimicrobial used. The wounds were dressed using topical agent and autoclaved banana leaf dressing. Large full thickness burn wounds, infected and macerated wounds were treated by exposure with application of povidone iodine ointment twice a day. The wounds were inspected during change of dressing and swabs were studied twice weekly until all burn wounds healed. During direct patient contact protective gown, mask and disposable gloves were used. Hands were washed with conventional soap and disinfected with 70% ethanol before and after patient contact.

Fungal growth was suspected in wounds that were macerated and had mossy blinded manuscript appearance with whitish patches. Punch biopsy from these wounds were taken and examined by 10% potassium hydroxide (KOH) mount, nigrosin stain, 1% acid fast bacilli (AFB) stain, Gomori methenamine silver (GMS) stain, histopathological examination and fungal culture. All such wounds were treated topically with miconazole ointment mixed with povidine iodine and itraconazole (oral) systemically. Specimens for fungal culture were collected as a piece of tissue including a part of unburnt tissue in depth (nearly 0.5 g in weight) in all cases. Sabouraud dextrose agar (SDA) was used for culture media while Corn meal agar (CMA) tween 80 and Cnisapek dox agar (CDA) were used as subculture media for isolation of fungi. Bacterial cultures were also performed for all patients. Facilities to perform antifungal sensitivity after obtaining fungal culture were not available. Statistical variables included days for burn wound closure, duration of antibiotic therapy and treatment with imipenem, vancomycin, linezolid. Outcome measures were length of hospital stay (LOS) and mortality. Statistical analysis was done using Chi-square test and student t-test. Sensitivity and specificity of the investigation performed was also worked out.

Results

Two hundred and one cases were treated as in-patients at the burns unit of LTM General Hospital, Mumbai, India during the period of study. Eighty percent of 201 patients had sustained flame burns, 12% had suffered from scalds and 2% had sustained electrical burns. During hospital stay, colonisation of burn wound with fungi was clinically suspected in 77 (38.31%) out of 201 patients (Table 1). The epidemiological data of these 201 patients is presented in Table 2.

Table 1 Demographics of burn patients with and without clinical suspicion of fungal colonisation

	Patients with suspicion (n = 77)	Patients without suspicion (n = 124)	p value
Age	29.75 ± 11.31	27.74 ± 10.72*	0.2073
TBSA%	48.40 ± 15.03	40.16 ± 15.07*	0.0002**
DBSA%	28.09 ± 10.57	20.85 ± 11.63*	<0.0001**

* Mean ± Standard deviation (SD)

** Statistically Significant

TBSA: total body surface area; DBSA: deep burn surface area

Patients with confirmed fungal colonisation had larger extent of burn and prolonged hospital stay ($p < 0.05$, statistically significant). Ninety-two samples were sent from 77 patients suspected to have fungal colonisation of burn wound. On investigations, fungal colonisation was confirmed in 35 patients. GMS stain and 10% KOH stain were positive in 25 patients and showed 70.8% sensitivity and 86.5% specificity (Table 3).

Nigrosin stain was negative in all patients revealing no capsulated fungi in the study group. One smear was AFB positive revealing nocardia infection. On culture, 24 isolates of fungi were identified in 23 patients with 11 *Candida albicans*, 1 *Candida tropicalis*, 3 *Aspergillus fumigatus*, 1 *Aspergillus flavus*, 2 *Fusarium* and 6 other fungi like 2 penicillium, 2 *Zygomycete*, 2 *Wangiella dermatitis*, etc.

Histopathological examination of wound biopsy of three patients revealed invasive fungal infection, all with 2b penetration (i.e. generalised invasion of viable tissue). Culture of these three samples failed to grow fungi. One fungal isolate was detected in burn wound in 1st week, 16 in 2nd week, eight in 3rd week, one in 4th week and 10 after 4th week. Associated bacterial colonisation was with *pseudomonas* in 22% of cases, methicillin resistant *Staphylococcus aureus* (MRSA) in 25%, *pseudomonas* and MRSA in 28% and others in 25%. Wounds with fungal colonisation required 33.24 days for obtaining wound bed suitable for closure with autograft, while wounds without fungal colonisation required 25.56 days ($p < 0.05$; statistically significant). Patients with suspicion of fungal wound colonisation required 35 days (average) of treatment with systemic antibiotics, where as patients without suspicion of fungal colonisation required 24 days of treatment with systemic antibiotics during their hospital stay ($p < 0.01$; statistically significant).

Out of 63 patients receiving imipenem, vancomycin, linezolid, 17 patients (26.98%) developed fungal colonisation and out of 138 patients without above antimicrobials, 18 patients (13.04%) developed fungal colonisation in their burn wounds ($p < 0.02$; statistically significant). Fungal infection was more common in patients treated with exposure of wounds (26.25%) than with closed dressing (11.57%) ($p < 0.01$; statistically significant) (Table 4).

Table 2 Characteristics of burn patients with and without confirmed fungal colonisation

	Fungal colonisation	No fungal colonisation	Total	p value
No. of patients	35	166	201	
Age	30.97 ± 11.88	27.99 ± 10.73	28.51 ± 10.97	0.1445
Sex	6 M, 29 F	37 M, 129 F	43 M, 158F	0.499
TBSA%	50.29 ± 16.08	41.85 ± 15.07	43.32 ± 15.55	0.0033
DBSA%	28.86 ± 10.30	22.52 ± 11.77	23.62 ± 11.75	0.0035
Hospital stay	35.37 ± 14.95	25.07 ± 15.90	26.87 ± 16.19	0.0005
Mortality%	25.71	25.30	25.37	0.96

TBSA: total body surface area; DBSA: deep burn surface area

Table 3 Outcome of investigations in patients with clinically suspected fungal colonisation

	Culture positive	Culture negative	Total No. of patients
Smear positive (KOH, GMS)	17	8	25
Smear negative	7	45	52
Total	24	53	77

Table 4 Association of fungal colonisation and type of wound care

	Exposure treatment (n = 80)	Closed dressing (n = 121)
TBSA%	48.40 ± 15.03*	40.16 ± 15.07*
DBSA%	28.09 ± 10.57*	20.85 ± 11.63*
Confirmed fungal colonisation	21 (26.25%)	14 (11.57%)
<i>Candida</i> isolates	8	4
<i>Aspergillus</i>	2	2

*Mean ± standard deviation (SD)

Discussion

Burn wound provides a suitable substrate for fungal colonisation, the reported incidences being around 20% [1–3]. Indian data is scanty. In our study, fungal colonisation was clinically suspected in 77 patients (out of 201 patients) and was confirmed in 35 patients. Clinical suspicion was more often associated with larger total extent of burn and extent of full thickness burn (Table 1).

Literature reports that frequency of fungal colonisation increases steadily during hospital stay peaking at third and fourth weeks [2]. In our study colonisation with fungi was more commonly seen in second and third week. Early and specific diagnosis is vital and the decision to treat such a patient has to be based on clinical and mycological information. Growth of fungal colonies is slow and may

take up to 3 weeks. The limitations associated with classic culture techniques for the diagnosis of invasive fungal infections have lead to the emergence of many non-culture based methods including deoxyribose nucleic acid (DNA)-based enzyme linked immunosorbent assay and polymerase chain reaction [4, 5]. These tests are not used widely and are not easily available in India. In our study, smear tests showed acceptable sensitivity and specificity. This has led us to suggest that smear test can be relied upon to initiate empirical anti-fungal treatment to curtail delay in confirmation with culture. Di Lonardo et al. found fungal infection on histopathological examination in 16 out of 176 biopsy samples from 40 patients [6]. McManus et al. while comparing quantitative microbiology and histopathology in divided burn-wound biopsy specimens found agreement of 96.1% between negative cultures and histologic absence of invasive infection. In sharp contrast, however, histologic invasion occurred in only 36% of specimens with positive cultures [7]. It has been reported that correlation between histopathologic and culture identification of fungi is inconsistent [8]. In our study, all the three samples with histological evidence of invasion showed negative cultures.

Burn patients are known to harbour various species of *Candida* and *Candida albicans* is always considered as the most frequent pathogenic species causing nosocomial fungal infections in burn patients [3, 9–13]. However, recently other species of *Candida* (i.e. *C. tropicalis*) has emerged to be equally important in these patients [2, 10, 14, 15]. In our study, *C. tropicalis* was detected in 4% of isolates though *C. albicans* continued to dominate (46%). Unlike *C. albicans*, it is well known that *C. tropicalis* is not a commensal and is usually associated with development of deep seated infection [2, 10, 14, 15].

Aspergillus has been the next common fungus infecting burn wound [3, 9] Significant percentage of *Aspergillus* infection (16.66%) was found in our study. Unlike *Candida*, which lives on skin or is found in the gut, *Aspergillus* is frequently found in compost heaps, air vents, and airborne dust and is known to cause invasive fungal infection. In

75% of our patients, fungal colonisation was associated with multi-drug resistant MRSA and *pseudomonas* infection demanding treatment with newer generation of antibiotics like imipenem, vancomycin and linezolid. Cochran et al. while studying systemic fungal infection in burn patients found that patients with multiple sites of *Candida* were more likely to have received imipenem, vancomycin or an aminoglycoside [16].

In our study group, high incidence of fungal colonisation may be due prolonged use of antimicrobials, which is required for longer duration as we don't have policy to graft wounds early. This is due to excessive work-load, limited staff and non-availability of operation theatre for burns cases, frequently.

We observed significantly higher incidence of fungal colonisation with exposure treatment of burn wounds. This corresponds with available data. The closed dressing method is generally recommended for patients with burn wounds [3].

Once diagnosed, primary therapy for fungal infection relies heavily on three classes of antifungal agents - polyenes (amphotericin, nystatin), extended-spectrum azoles (fluconazole, itraconazole) and echinocandins (caspofungin). Although fluconazole is effective against *Candida*, it is important to note that this drug has no activity against molds, including *Aspergillus*. With the rising prevalence of this fungus, fluconazole can not be relied upon and we suggest that drugs like itraconazole, variconazole [4] need to be used as empirical antifungal therapy.

Serious strategies need to be employed to prevent and control fungal infections. As in the prevention of other infections, practical measures such as air filtration, regular hand washing and avoidance of potted plants and flowers needs to be adopted to avoid exposing patients to fungi [17].

Conclusion

In our study, the incidence of fungal colonisation of burn wounds was 38.31% on clinical suspicion and was confirmed on investigations in 17.41%. *Candida* and *Aspergillus* were the most commonly detected fungi. Smear tests were reliable with 73% sensitivity, 89% specificity and had advantage of being easy, cheap and fast. Fungal infection was more in patients with larger extent of burn. Hospital stay was more prolonged in patients with fungal colonisation but the mortality was not affected. We suggest use of extended spectrum antifungal agents, e.g. itraconazole in view of emergence of *non-Candida* fungi.

References

1. Ali Akayled AT (1999) Invasive Burn Wound Infection. *Ann Burns and Fire Disasters* 12(4):204–206
2. Jefferson Lessa Soares de Macedo, Joao Barberino Santos (2005) Bacterial and Fungal colonisation of Burn wounds. *Mem Inst Oswaldo Cruz*. Rio de Janeiro 100(5):535–539
3. Mousa HA (1999) Fungal infection of burn wound in open and occlusive treatment methods. *East Mediterr Health J* 5:333–336
4. Siew Fah Yeo, Brian Wong (2002) Current status of Non culture methods for diagnosis of Invasive Fungal infections. *Clin Microbiol reviews* 15(3):465–484
5. White P Lewis, Archer Alice E, Barnes Rosemary A (2005) Comparison of Non-Culture-based methods for detection of Systemic Fungal Infections, with emphasis on Invasive *Candida* infections. *J Clin Microbio* 43(5):2181–2187
6. Di Lonardo A, Ferrante M, Maggio G, et al. (1993) Histological assessment of the level of burn wound infection: Diagnostic and Therapeutic Strategies. *Ann Medit Burns Club* 6(2):90–93
7. McManus AT, Kim SH, McManus WF, Mason AD, Pruitt BA (1987) Comparison of quantitative microbiology and histopathology in divided burn-wound biopsy specimens. *Arch Surg* 122(1):74–76
8. Schofield CM, Murray CK, Horvath EE, et al. (2007) Correlation of culture with histopathology in fungal burn wound colonisation and infection. *Burns* 33(3):341–346
9. Chakrabarti A, Nayak N, Kumar PS, et al. (1992) Surveillance of nosocomial fungal infection in burn care unit. *Infection* 20:132–135
10. Gupta N, Haque A, Lattif Ali Abdul, et al. (2004) Epidemiology and molecular typing of *Candida* isolates from burn patients. *Mycopathologia* 158:397–405
11. Macdeo JLS (2003) Immunodepressao do queimado: patogenese e fator de risco para sepsis. *Rev Bras queimadura* 3:26–35
12. Mousa HA (2003) Anaerobic, aerobic and fungal burn wound infections. *J Hosp Infect* 37: 317–323
13. Smith Jr DJ, Thompson PD (1992) Changing flora in burn and trauma units: historical perspective – experience in the United States. *J Burn Care Rehabilitation* 13:276–280
14. Leung AY, Chim CS, Ho PL, et al. (2002) *Candida tropicalis* fungaemia in adult patients with haematological malignancies: clinical features and risk factors. *J Hosp Infect* 50:316–319
15. Mathews MS, Samuel PR, Suresh M (2001) Emergence of *Candida tropicalis* as the major cause of fungaemia in India. *Mycoses* 44:278–280
16. Cochran A, Morris SE, Edelman LS, Saffle JR (2002) Systemic *Candida* infection in burn patients: A case-control study of management patterns and outcomes. *Surg Infect (Larchmt)* 3(4):367–374
17. Chandrashekar PH (2006) Serious fungal infection: New strategies to optimize treatment outcomes. First Report: AACME and Princeton Media Associates Dec:3–7