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Effect of Burn Injury on the Dissemination of *Candida albicans* from the Skin of Mouse

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ABSTRACT

Burn injury is associated with the greatly increased susceptibility of thermally injured patients to infection from a variety of pathogens. *Candida albicans* **is a commensal of skin and has been reported as a severe pathogen in thermally injured patients. Systemic candidiasis has emerged as a major cause of death in burn patients. In this study, the ability of** *Candida albicans* **to infect thermally injured host and the host response to systemic infections with** *Candida albicans* **was studied by using burned mice. The** *C. albicans* **suspension was spreaded over the burn surface. Mice were sacrificed ten days after burn to check the presence of yeasts in the kidneys, lung, liver and spleen or burn wounds. After 48 hr. of incubation of culture plates yeasts were recovered from the kidneys, lung, liver, spleen of burned mice. This study describes the enhanced susceptibility of burned mice to systemic candidiasis and shows that a systemic infection with Candida can lead organisms to contaminate the wound.**

Keywords: Burn patients, *Candida albicans*, Candidiasis, Swiss albino mice, Thermal injury.

INTRODUCTION

The genus Candida includes about 150 different species, however, only a few are known to cause human infections. *C. albicans*, is a normal constituent of the human flora, a commensal of the skin and the gastrointestinal and genitourinary tracts. *C. albicans* is the most significant pathogenic specie which can cause infections [called candidiasis or thrush] in humans and other animals (Kaminski *et al*., 1995). Other Candida species pathogenic in humans include *C. tropicalis, C. glabrata, C. krusei, C. parapsilosis, C. dubliniensis*, and *C. lusitaniae* (Fader *et al*., 1985). *C. albicans* remains the most common infective species, other *Candida spp*. are becoming increasingly significant. In a range of large-scale studies of candidaemia between 1999 and 2006, about 52% of 9717 cases involved *C. albicans*, about 30% involved either *C. glabrata* or *C. parapsilosis* and less than 15% involved *C. tropicalis, C. krusei* or *C. guilliermondii* (Marissa *et al*., 2012). Candidiasis is a common infection of the skin, oral cavity and esophagus, gastrointestinal tract, vagina and vascular system of humans. Most infections occur in immunocompromised patients. *C. albicans*, expresses several virulence factors that contribute to pathogenesis. These factors include host recognition biomolecules (adhesions), morphogenesis (the reversible transition between unicellular yeast cells and filamentous, growth forms), phenotypic switching, secreted aspartyl proteases and phospholipases (Murciano *et al*., 2006). *C. albicans* is a dimorphic fungus. Normal room temperatures favor the yeast form of the organism, but under physiological conditions (body temperature, pH and the presence of serum) it may develop into a hyphal form. Pseudohyphae, composed of chains of cells, are also common. However, the ability to assume various forms may be related to the pathogenicity of the organism. The yeast form is 10-12 µm in diameter. Pseudohyphae [chains of cells] may be formed from budding yeast cells which remain attached to each other. Chlamydospores may be formed on the pseudomycelium (Calum *et al*., 2009). To infect host tissue, the usual unicellular yeast-like form of *C. albicans* reacts to environmental cues and switches

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into an invasive, multicellular filamentous form. The ability of *C. albicans* to form hyphae has been proposed as a virulence factor as these structures are often observed invading tissue and *C. albicans* strains which are unable to form hyphae are defective in causing infection. Although Candida most frequently infects the skin and mucosal surfaces, it can cause systemic infections manifesting as pneumonia, septicaemia or endocarditis in severely immunocompromised patients. Systemic fungal infections [fungemias] have emerged as important cause of morbidity and mortality in immunocompromised patients.

Burn injury is associated with the greatly increased susceptibility of thermally injured patients to infection from a variety of pathogens (Kobayashi *et al*., 1998) compared with other trauma patients, because of loss of the skin barrier (Ruth, 1980). Skin integrity is of importance for the protection and separation of body tissues from the surrounding environment. The loss of skin due to burns or trauma exposes the body to severe stress; impairing or even eliminating the many vital functions this organ performs (Alice *et al*., 1985). *C. albicans* has been reported as a severe pathogen in thermally injured patients (Kobayashi *et al*., 1998).The pathogenesis of fatal Candida infections in burn patients is complicated (Alice *et al*., 1985). Severe burn injury induces an immunosuppressive state which is responsible for the increased susceptibility to development of opportunistic infections that can lead to sepsis and increase mortality (Patenaude *et al*., 2010). The susceptibility of thermally injured mice to *C. albicans* infection was 50 times greater when compared to the sensitivity of normal mice (Kobayashi *et al*., 1998). Burn injury leads to suppression of nearly all aspects of immune response. Chemotaxis, phagocytosis, and killing function of neutrophils, monocytes, and macrophages are impaired. Systemic candidiasis and chronic mucocutaneous candidiasis are common infections in burn patients (Calderone and Fonzi, 2001). Systemic candidiasis has emerged as a major cause of death in burn patients. Host defense against systemic candidiasis relies mainly on the ingestion and elimination of *C. albicans* by cells of the innate immune system, in particular macrophages, monocytes and neutrophils. Increased incidence of infection is due to defects in non-specific defense functions, such as neutrophil and macrophage phagocytosis and bacterial activity, as well as to an inhibition of specific immune functions, such as cell-mediated immunity. The decrease in host resistance to infection is obviously related to a depression of both humoral and cellular components of the host defense system (Okawa *et al*., 2004).

The present study was performed by burning the skin of mouse and investigating the dissemination of *C. albicans* from skin to different organs of the mouse.

MATERIALS AND METHODS

Organisms: The *C. albicans* was maintained on Sabouraud dextrose agar slants at 4°C. To prepare cultures for animal inoculation, the stock culture was inoculated into 25 ml of Sabouraud dextrose broth and incubated for 48 hr at 37°C in an environmental shaker incubator at 125 rpm. The organisms were harvested by centrifugation and suspended to a concentration of 10^8 organisms per ml in sterile saline.

Mouse burn model: Swiss albino mice of either sex weighing 20 to 25 g. The animals were acclimated for 1 week before burning and given water and standard laboratory chow ad libitum throughout the study. The mouse was anesthetized with chloroform. The back of the mice was shaved, and the animals were anesthetized with chloroform. Ethanol (0.5 ml) was evenly spread over the area of the back, ignited, and allowed to burn for 10 sec. The animals were given 1 ml of normal saline intraperitoneally as fluid replacement therapy. This method, which produced a partial-thickness burn of approximately 30% of the body surface, was not fatal for any of the animals. Animal inoculation consisted of 0.1 ml of the Candida suspension (10^8 organisms per ml) spread onto the surface of the wound. The animal was sacrificed after 10 days post burn. Biopsies were obtained for quantitation of organisms in the tissue.

Quantitation of organisms in tissue: Biopsies (skin,

wound, or kidneys) were weighed and homogenized in tissue grinders containing 5 ml of sterile saline. The samples were diluted in saline and 0.1-ml portions were plated in duplicate by the agar pour plate technique. Sabouraud dextrose agar containing chloramphenicol to suppress bacterial growth was used as the growth medium. The plates were incubated for 48 hr at 37°C, and colonies were counted. CFU/g of tissue was determined by the following formula: [(mean no. of CFU/ml)/ (weight of biopsy)] x [(5 ml)/ (dilution)].

RESULTS AND DISCUSSION

Candidiasis is the most common fungal infectious disease in burn patients. Colonization of *C. albicans* in burn patients is found to be very high i.e., about 63% (MacMillan *et al.*, 1972). The present investigation was undertaken to study the dissemination ability of *C. albicans* by using thermally injured mouse as possible animal model of infection. The study of Alice *et al* (Alice *et al.*,1985). initially described the enhanced susceptibility of thermally injured mice to an intravenous injection of Candida. Concerning the depth of burn, our previous study demonstrated that a deeper burn increased the susceptibility of mice to wound invasion after topical application of Candida (Avniel *et al.*, 2006). This finding agreed with a study on Candida infections in burn patients, which report that Candida invaded full- thickness wounds more often than partial-thickness burns (Kobayashi *et al.*, 1998). The deeper burn in our model may account for the apparent increased mortality we observed at lower concentrations of Candida, although the strain of mouse used may also have affected the outcome of infection. The result of the studies emphasize the importance of a careful strain selection when doing animal studies, and each study describes an animal model that can be used to study the effect of thermal injury on the pathogenesis of candidiasis (Fader *et al.*, 1986).

The morbidity and mortality of burned mice in response to systemic infection with *C. albicans* were examined in mice given a burn and suspension of *C. albicans* by spreading over the surface of wound. The scald burn, of 7 sec duration, resulted in a uniform full-thickness injury, at ten days after infection, the kidneys, wound, liver ,lung, spleen or skin were assayed for the presence of the organisms. In burned mice, organisms were uniformly isolated from the organs at the time of sacrifice (Table I and II). *C. albicans* that was spread over the surface of the wound disseminate quickly and large number of colonies was observed on culture media by Culture different organs of the burned mice (Figure 1-7).

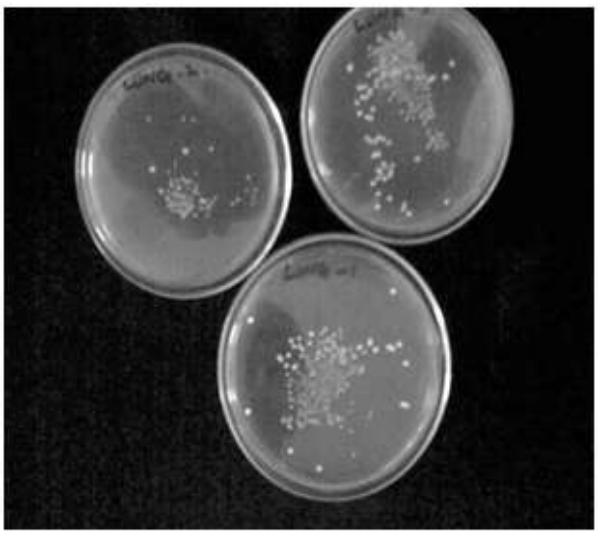

Figure 1. Culture from lung sample after 24hr.

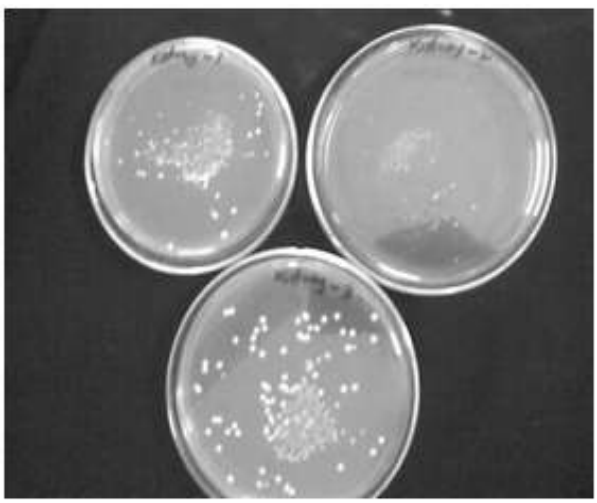

Figure 2. Culture from kidney sample after 24hr.

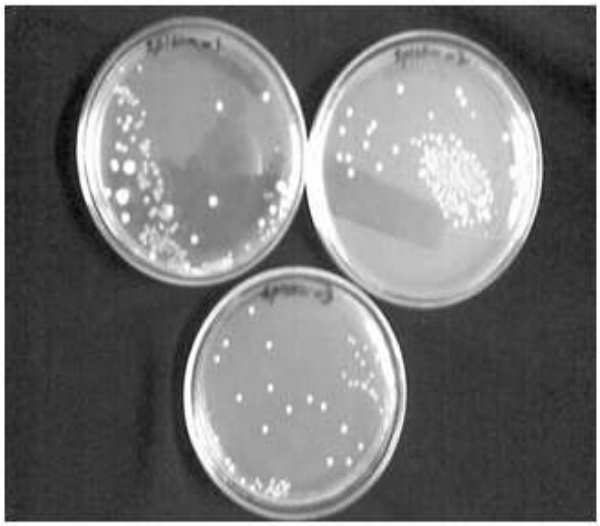

Figure 3. Culture from liver sample after 24hr.

Figure 4. Culture from spleen sample after 24hr.

Table I. Isolation of *C.albicans* from tissue after 24 hrs of incubation. kidney sample after 48 hr.

Organ/Tissue	No. of colonies		
	1st plate	2nd plate	3rd plate
Lung	204	100	84
Liver	320	96	120
Kidney	140	90	144
Spleen	132	140	146

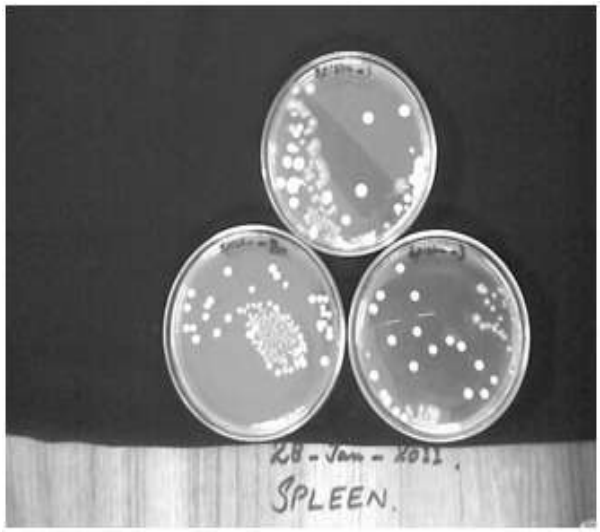

Figure 5. Culture from spleen sample after 48 hr.

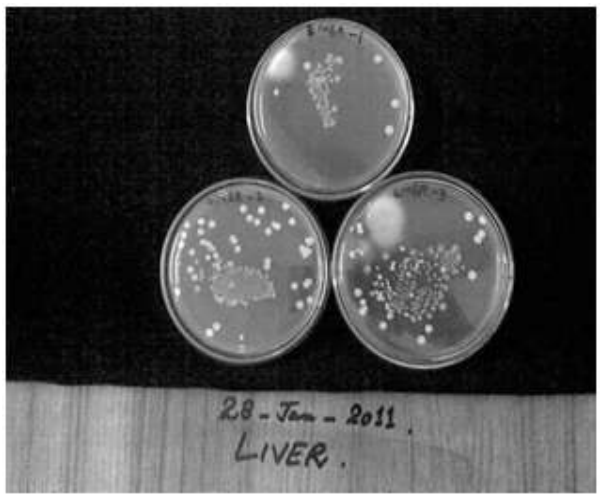

Figure 6. Culture from liver sample after 48 hr.

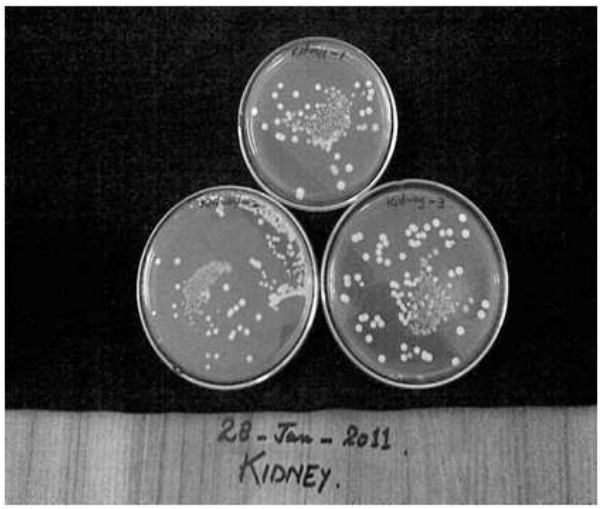

Figure 7. Culture from kidney sample after 48 hr.

Table II. Isolation of *C.albicans* from tissue after 48 hrs of incubation.

Organ/Tissue	No. of colonies		
	1st plate	2nd plate	3rd plate
Lung	360	140	108
Liver	52	148	280
Kidney	280	160	128
Spleen	200	160	116

Organ Culture revealed that the organisms arrived in the organ via the blood stream. In a previous study the mice were injected via an intraperitoneal injection, contiguous spread from the peritoneum was not observed as no yeasts were observed in the lower skeletal muscle of the dorsum (Okawa *et al*., 2004). This study demonstrated that thermal injury enhances the ability of *C. albicans* to infect mice and that the depth of burn appears to be an important factor in determining whether the organisms can invade the burn wound to cause systemic infection. This animal model should be valuable in elucidating the virulence factors of *C. albicans* that play a role in the pathogenesis of candidiasis after thermal injury.

REFERENCES

Alice NN, Law EJ and Holder IA.1985.Increased Susceptibility To Lethal Candida Infections In Burned Mice Preinfected With Pseudomonas.Aeruginosa Or Pretreated With Proteolytic Enzymes. Infect Immun, 49:780-784.

Avniel S, Arik Z, Maly A, Sagie A, Basst HB, Yahana HD,*et al*. 2006. Involvement Of The Cxcl12/Cxcr4 Pathway In The Recovery Of Skin Following Burns. J Invest Dermatol., 126:468-476.

Calderone RA and Fonzi WA. 2001. Virulence Factors Of Candida.Albicans. Trends, 9:327-35.

Calum H, Moser C, Jensen PO, Christophersen J, Maling DS,Van Gennip M.2009. Thermal Injury Induces Impaired Function In Polymorphonuclear Neutrophil Granulocytes And Reduced Control Of Burn Wound Infection. Clin Exp Immunol., 156: 102–110.

Fader RC, Nunez D, Unbehagen J, and Linares HA.1985. Experimental Candidiasis After Thermal Injury. Infect. Immun., 49: 780-784.

Faderr C, Carlile JC, Unbehagen J and Linares HA.1986.Systemic Candidiasis After Thermal Injury. Curr Microbiol,14: 231-234.

Kaminski H, Miyaguchi H, Tamaki T, Suenaga N, Hisamatsu M, Mihashi I, *et al*. 1995. Degradation Of Humoral Host Defense By Candida albicans Pro teinase. Infect. Immun., 63:984-988.

Kobayashi M, Kobayashi H, Herndon DN, Pollard RB and Suzuki F.1998. Burn-Associated Candida.Albicans Infection Caused By Cd30+Type 2 T Cells. J. Leukoc. Biol., 63: 723-731.

Macmillan BG, Law EJ and Holder IA. 1972. Experience with Candida Infection In The Burn Patient. Arch. Surg., 104:509-514.

Marissa PS, Tristram S, and Brown S. 2012. The Contribution Of Growth Rate To The Pathogenicity Of Candida Spp. Inter J Biol Life Scie, 8:80-86.

Murciano C, Villamón E, Yáñez A, O'connor JE, Gozalbo D and Gil ML .2006. Impaired Immune Response To Candida.Albicans In Aged Mice. J Med Microbiol, 55: 1649-1656.

Okawa Y, Kobayashi M, Sakai K and Suzuki M.2004. Role Of Polymorphonuclear Leukocytes In The Resistance Of Tumor-Bearing Mice Against Candida.Albicans Infection. Biol. Pharm. Bull., 27: 674—678.

Patenaude.J, Elia MD, Hamelin C, Bernie J.2010. Selective Effect Of Burn Injury On Splenic Cd11c+ Dendritic Cells And Cd8a+Cd42cd11c+ Dendritic Cell Subsets. Cell. Mol. Life Sci., 67:1315–1329.

Ruth E.1980. In Vitro Phagocytosis Of Candida.Albicans By Peritoneal Mouse Macrophages.Infect.Immun., 28:963-971.