Pathological Studies on Experimental Systemic Candidiasis induced by Candida albicans Isolated from Different Animals in Immunosuppressed Mice

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Abstract: Fifty male mice (6 weeks old) were used to evaluate the severity of the pathological lesions induced by Candida albicans isolated from different animals (goats, sheep, cattle and buffaloes). The mice were immune suppressed by subcutaneous injection of 0.5 mg cortisone/kg B wt for 5 successive days before the beginning of the experiment and extended to the first 5 days after Candida albicans inoculation. These mice were randomly assigned to five groups (n=10). These groups intravenously (via tail vein) inoculated with 0.5 ml suspension of candida albicans 1 x 10⁶ blastospores isolated from goats (gp 1), sheep (gp 2), cattle, (gp3) or buffaloes (gp 4), besides the gp (5) which inoculated with phosphate buffer solution (PBS) as a control group. The clinical signs, mortalities and the gross lesions were recorded before different specimens from lungs, heart, liver, kidneys, spleen and brain collected and were routinely processed for histopathological examination.Multiple granulomas were detected replacing the pulmonary tissue, pleura, myocardium, hepatic and renal parenchyma of gps (1 and 2). Such granulomas were represented by central basophilic structurless mass containing blastospores, pseudohyphae, hyphae and oval yeast cells, 3-8 µm in diameter, surrounded by a thick zone of mononuclears mostly of macrophages and lymphocytes besides few polymorphnuclear cells. Fibrinonecrotic pseudomembranes and multifocal suppurative areas were observed in the pleura and pericardium. Meanwhile, the gps (3 and 4) showed minimal lesions and poor fungal growth besides lowering in mortalities from 70-80% (gps 1 and 2) to 30-40% (gps 3 and 4). Finally, it could be concluded that the Candida albicans, isolated from goats and sheep, induced severe multiple lesions than that isolated from cattle and buffaloes.

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1. Introduction

Candidiasis is a disease caused by a dimorphic Candida sp. which is part of the normal flora found in the upper respiratory, gastrointestinal and female genital tract of the human body (Brawner and Cutler 1989 and Kumamoto and Vinces 2005). Most cases of Candida infection result from *Candida albicans*, which is an opportunistic infection as it does not induce disease in immunocompetent individuals but can only do so in those with impaired host immune defenses (De Repentigny et al 1992 and Jarvis and Martone 1992).

Nowadays, the incidence of invasive fungal infection has been increasing, mostly due to advances in medicine that may produce immunocompromised individuals (Nakayama et al 2010). Candida species are implicated in cases of superficial and disseminated candidiasis due to the administration of broad-spectrum antibiotics, corticosteroids and immunosuppressive drugs (Fraser et al 1992 and Ozcan et al 2006). Diabetes mellitus, viral infections, and urinary and venous catheters were also reported as risk factors (Hamir et al 2000 and Pressler et al 2003) and the patients are often resistant to conventional antifungal therapy and may cause high morbidity and mortality rates (Morgan 2005 and Spellberg et al 2006) . *Candida* pathogen was accounted for approximately 50 to 60% of all *Candida* blood culture isolates (Wisplinghoff et al 2004) and by far the most common species causing infections in humans (Vazquez and Sobel 2002) and can invade and damage a wide range of host tissues during systemic infections that often results in death, even in patients treated with antifungal agents (Roilides et al 2003).

Omuta et al (2007) reported that the systemic candidiasis tends to cause lesions in the lungs, kidneys, heart, spleen and brain. *C. albicans* infection was assessed by evidence of lesions and by presence of hyphae on the affected organs (O'Grady and Reade 1993). Multiple pyogranulomatous lesions with blastospores, pseudohyphae, and true hyphae of *Candida albicans* were observed in various organs in dogs with systemic candidiasis (Tunca et al 2006 and Matsuda et al 2009). Hosogi et al (2008) observed apoptosis of alveolar epithelial cells in mice that given an intravenous injection of Candida albicans inducing acute lung injury. Ashman (1998) reported that the mouse model of acute infection with C. *albicans* is a valuable experimental model for studying microbial pathogenesis, as it includes many of the clinical features of the human condition.

The objective of this work was to evaluate the severity of the pathological lesions of *Candida albicans* isolated from different animals species (goats, sheep, cattle and buffaloes).

2. Material and Methods

Animals:

Fifty male mice (6 weeks old and weighed 20 to 25 gm) were obtained from the Unit of Laboratory Animal, Faculty of veterinary medicine, Zagazig University, Egypt. Mice were placed in polycarbonate cages with stainless-steel wire tops and maintained at 24 to 26°C with 55 to 75% humidity and a 12-h light/dark cycle, and fed a commercial rodent diet and given water ad libitum. The mice received humane care. They were immune suppressed by subcutaneous injection of 0.5 mg cortisone/kg B wt[•] for 5 successive days before the beginning of the experiment and extended to the first 5 days after Candida albicans inoculation (Kamai et al 2001).

Source and Culture of Fungal Strains

Samples obtained from the tongues with lesions of goats, sheep, cattle and buffaloes (table,1) were plated directly in Sabouraud dextrose agar (Difco Laboratories, Detroit, MI, USA) added with chloramphenicol (0.1 mg/ml), and incubated at 37° C for 48 h (Cruickshank et al 1975 and Freire-Garabal et al 1999). The isolated organisms were identified as *C. albicans* by the germ tube test, chlamydospore production as described by Schaar et al (1974) and

the API 20 C AUX kit "biomérieux, Marcy-L'Etoile, France" (Nowotny 1979 and Sandvén 1990).

Table (1): Source	and	number	of	total	and	positive
sample						

Animal Source	No. of sample	No. of positive sample	%
Goats	28	16	57.14
Sheep	25	14	56
Cattle	23	13	56.52
Buffaloes	26	11	42.31
Total	102	54	52.94

Inoculum preparation

Candida albicans isolated from the different animals were subcultured on Sabouraud dextrose agar and incubated at 37°C for 3 days. Loopful from the growth was suspended in phosphate buffer solution (PBS) and compared with MacFarlane number 3 for turbidity containing 1 x 10^6 cfu/ml (Hoyer et al 1999).

Mice inoculation

On day 6 after cortisone injection, these mice were randomly assigned to five groups (n=10). These groups intravenously (via tail vein) inoculated with 0.5 ml suspension of 1×10^6 Candida albicans *blastospores* isolated from goats (gp 1), sheep (gp 2), cattle, (gp3) or buffaloes (gp 4), besides the gp (5) which inoculated with phosphate buffer solution (PBS) as a control group (table, 2).

Table (2): Mice groups, number of mice, treatment and number of dead and sacrificed mice.

Groups	No. of mice	Treatment 1x10 ⁶ blastospores of	No. of dead mice within 8-14 day post inoculation	No. of sacrificed mice on 15 th day post inoculation
1	10	C. albicans from goats	7	3
2	10	C. albicans from sheep	8	2
3	10	C. albicans from cattle	4	6
4	10	C. albicans from buffaloes	3	7
5	10	Control (PBS injection)	0	10
Total	50		22	28

• Fortcortin contain 8 mg/2 ml dexamethasone, Merck-Germany

Pathological Examination

The clinical signs and mortality rate were evaluated. At the end of the experiment, all remaining mice were sacrificed for complete necropsy and all macroscopic abnormalities were recorded in *C. albicans*-infected mice in each group. Specimens from the lungs, heart, liver, kidneys, spleen and brain were collected and fixed in 10% neutral buffered formalin solution. Five micron thick paraffin sections were prepared and stained by hematoxylin and eosin (HE) and periodic acid-Schiff (PAS) for histopathological examinations (Bancroft and Stevens 1996).

3. Results

Clinical Signs and Mortality rates:

Dyspnea, anorexia and distended abdomen besides emaciation and ruffled hair coat were the most common clinical signs of the *C. albicans* particularly that isolated from goats and sheep. The mortalities were high in gps 1 and 2 (70% and 80%), respectively and they were low in mice of gps 3 and 4 (40% and 30%), respectively.

Pathological Findings:

Macroscopically, the mice infected with *C*. *albicans* isolated from goats (gp 1) or from sheep gp (2) showed yellowish-white mottled lungs with numerous red spots 1-3 mm in diameter. Multifocal pale yellow foci on the liver (Fig 1), the epicardium and the adjacent myocardium were noticed.

Microscopically, multiple granulomas were detected replacing the pulmonary tissue (Fig 2), pleura (Fig 3), myocardium (Fig 4), hepatic (Fig 5) and renal (Fig 6) parenchyma. Such granulomas were represented by central basophilic structurless mass containing blastospores, pseudohyphae, hyphae and oval yeast cells, $3-8 \ \mu m$ in diameter, surrounded by a thick zone of mononuclears mostly of macrophages and lymphocytes besides few polymorphnuclear cells (Fig 7). The fungal elements were stained pink by PAS reaction (Fig 8).

Fibrinonecrotic pseudomembranes and multifocal suppurative areas were observed in the pleura (Fig 9) and pericardium (Figs 10 and 11). Basophilic suppurative emboli in the perialveolar (Fig 12) and peritubular (Fig 13) capillaries were detected in the lungs and kidneys; respectively besides myomalacia cordis in the myocardium (Fig 14). Interstitial aggregations of mononuclears were seen in the kidneys (Fig 15), liver and heart. Periportal hepatocytes showed coagulative necrosis, represented by pyknosis and karyorrhexis (Fig 16), and the cytoplasm of some necrotic hepatocytes showed refractile eosinophilic granules (Fig 17). Congestion, hemorrhage and degenerative changes (Fig 18) besides cellular casts were visualized in the affected kidneys (Fig 19). Sometimes, the granulomas revealed numerous neutrophils with minimal fibroblastic proliferation, particularly in the lungs and kidneys (Fig 20). Zenker's necrosis and edema with widely separated cardiac muscle fibers were noticed in the heart of most infected cases.

Focal and mild microscopic lesions were also seen in the brain and spleen. The brain showed edema in the Virchow Robin spaces and ventricles besides some degenerated neurons, satellitosis and neuronophagia (Fig 21). Few scattered neutrophils were observed infiltrating the pia mater. Meanwhile, the spleen revealed significant depletion in the lymphocytes of white pulp and hemosiderosis in the red pulp (Fig 22).

Meanwhile, the mice infected with *C. albicans* isolated from cattle (gp 3) or from buffalo (gp 4) showed congested and edematous lungs. The liver and other organs were moderately congested. The described lesions were mostly minimal with poor fungal growth (blastospores and budding cells) in the tissue comparing with the previous groups. The lungs showed focal thickening of the interalveolar septa with septal cells proliferation and polymorphnuclear cell infiltrations. Bronchiolitis with desquamated lining epithelium, severe congested blood vessels and inflammatory cells infiltration was also seen (Fig 23).

Perivascular and alveolar edema were visualized. The heart revealed thickening of the pericardium with edema infiltrated with few neutrophils (Fig 24). Such edema was extended to the myocardium represented by widely separated cardiac muscles fibers and around the cardiac blood vessels (Fig 25). The liver showed congestion of the hepatic blood vessels and sinusoids (Fig 26) with few round cells infiltrating the portal areas. Small areas of coagulative necrosis were rarely encountered. The kidneys showed congestion of the renal blood vessels and hemorrhage among the renal tubules (Figs 27 and 28). Some renal tubular epithelia showed hydropic degeneration and coagulative necrosis. Focal interstitial aggregations of leukocytes predominantly neutrophils were seen.



Figs (1-6): Groups (1 and 2) are similar.

Liver showing multifocal pale yellow foci (1).

Multiple granulomas replacing the pulmonary tissue ,HE x300 (2), pleura, HE x300 (3),myocardium, HE x1200 (4),hepatic, HE x300 (5) and renal parenchyma, HE x300 (6).



Figs (7-12): Groups (1 and 2) are similar.

Heart showing blastospores, pseudohyphae and hyphae among the fragmented cardiomyocytes, HEx3000 (7). The blastospores were stained pink by PAS stain x3000 (8).

Fibrinonecrotic pseudomembranes and multifocal suppurative areas were observed in the pleura, HEx1200 (9) and pericardium, HEx300 (10) and HEx1200 (11). Lung showing basophilic suppurative emboli in the perialveolar capillaries, HEx1200 (12).



Figs (13-18): Groups (1 and 2) are similar.

Kidney showing basophilic suppurative emboli in the peritubular capillaries, HEx1200 (13). Heart showing myomalacia cordis in the myocardium, HEx1200 (14). Kidney showing interstitial aggregations of mononuclears, HEx1200 (15). Liver showing coagulative necrosis of periportal hepatocytes, represented by pyknosis and karyorrhexis, HEx1200 (16), and the cytoplasm of some necrotic hepatocytes showed refractile eosinophilic granules, HEx3000 (17). Kidney showing congestion, hemorrhage and degenerative changes, HEx1200 (18).



Figs (19-22): Groups (1 and 2) are similar.

Kidney showing cellular casts inside the lumens of some renal tubules , HEx1200 (19). Lung showing granuloma with numerous neutrophils with minimal fibroblastic proliferation, HEx1200 (20). Brain showing some degenerated neurons, satellitosis and neuronophagia, HEx1200 (21). Spleen showing depletion in the lymphocytes of white pulp , HEx300 (22).

Figs (23&24): Groups (3 and 4) are similar

Lung showing bronchiolitis with desquamated lining epithelium, severe congested blood vessels and inflammatory cells infiltration, HEx300 (23). Heart showing thickening of the pericardium with edema infiltrated with few neutrophils, Hex1200 (24).



Figs (25-28): Groups (3 and 4) are similar

Heart showing edema represented by widely separated cardiac muscles fibers and around the cardiac blood vessels, HEx1200 (25). Liver showing congestion of the hepatic blood vessels and sinusoids, HEx1200 (26). The kidneys showed congestion of the renal blood vessels (27) and hemorrhage among the renal tubules, HEx1200 (28).

4. Discussions

It is evident that the C. albicans isolated from sheep and goats were the most pathogenic among the C. albicans isolated from the different animals (cattle and buffaloes) used in this study. They induced high mortalities among the examined mice of gps (1 and 2). Such high mortality (70 and 80%) may be due to the presence of large numbers of blastospores in the examined organs. Blastospores were more rapidly and consistently fatal to mice than the hyphae (Evans 1981). Allendoerfer et al (1993) found that a high-dose intravenous injection of C. albicans was associated with increased levels of TNF- and an increased mortality in mice. Scoring of candidiasis and its associated lesions in the lungs. heart, liver, kidneys, spleen and brain could explicate the pathogenesis of C. albicans in mice and its spread through the blood (Clancy et al 2000).

C. albicans isolated from sheep and goats multiple granulomas replacing induced the pulmonary tissue, pleura, myocardium, hepatic and parenchyma. Such granulomas renal were represented by central basophilic structurless mass containing blastospores, pseudohyphae, hyphae and yeast cells; surrounded by a thick zone of mononuclears mostly of macrophages and lymphocytes besides few polymorphnuclear cells. Fibrinonecrotic pseudomembranes and multifocal suppurative areas were observed in the pleura and pericardium. Basophilic suppurative emboli in the

perialveolar and peritubular capillaries were detected in the lungs and kidneys; respectively besides myomalacia cordis in the myocardium. Focal areas of coagulation necrosis, represented by pyknosis and karyorrhexis, and interstitial aggregations of mononuclears were seen in the kidneys, liver and heart. Congestion, hemorrhage and degenerative changes besides cellular casts were visualized in the affected kidneys. Zenker's necrosis and edema with widely separated cardiac muscle fibers were noticed in the heart of most infected cases. The brain showed edema in the Virchow Robin spaces and ventricles besides some degenerated neurons, satellitosis and neuronophagia. Meanwhile, the spleen revealed significant depletion in the lymphocytes of white pulp. Moreover, the significant correlation between the severities of lesions was accompanied by the C. albicans isolates, where it became mild and caused the least damage and, quite notably, formed fewer blastospores and budding cells in tissue of gps (3 and 4) and with low mortalities (30 and 40%).

The previous results are in agreement with the experimental studies conducted by Vose et al (2001), Feman et al (2002) and Tunca and Haz roglu (2004). They observed that systemic candidiasis tend to cause lesions and candida was observed in the lungs, kidneys, heart, liver spleen, myocardium, pericardium and brain. Tunca et al (2006) also observed multiple pyogranulomatous lesions besides blastospores, pseudohyphae, and true hyphae of

Candida albicans in various organs in male dogs with systemic candidiasis. The current study revealed that C. albicans isolated from sheep and goats induced suppurative pneumonia infiltrated by neutrophils, lymphocytes and some macrophages. Blastospores were also seen in the centers of the lesions. This result is disagreement with Trudeau and Saranac (1990) who found that the pulmonary tissue was quantitatively very resistant to C. albicans infection because of the ability of resistant pulmonary alveolar macrophage to rapid phagocytozed and kill yeast. Moreover, the presence of neutrophils in the pyogranulomatous lesions, could help the macrophages in the phagocytic process and play an important role in the prevention of fungal growth and the invasion of tissues. Defects in neutrophil number and function have been consistently implicated in the pathogenesis of disseminated candidiasis (Fradin et al 2005). Subsequently, neutropenia has been consistently implicated as a risk factor for the development of disseminated candidiasis (Hope et al 2002, Sallah et al 2001 and Uzun et al 2001). The exact mechanisms of pathogenesis of systemic candidiasis remain incompletely understood and no specific virulence factor is dominant (Calderone and Fonzi 2001). Rather, pathogenesis depends upon the coordinated expression of multiple genes in a manner that facilitates proliferation, invasion and tissue damage within the given in vivo milieu (Mahan et al 2000, Staib et al 2000 a and b and Fradin et al 2003). A number of C. albicans genes are likely to play roles in the pathogenesis of candidal disease at diverse tissue sites (Mahan et al 2000, Staib et al 2000 a and b and Fu et al 2002), however, other genes are likely to make distinct temporal-spatial contributions to virulence (Muhlschlege and Fonzi 1997 and De Bernardis et al 1998). Past studies have shown that C. albicans produces farnesol in vitro (Hornby et al 2001) and that increased production of farnesol in vivo is accompanied by increased virulence of C. albicans (Navarathna et al 2005). Hornby et al (2001) proposed that the farnesol excreted during infection would alter the membrane fluidity of host cells, allowing C. albicans to penetrate host tissues and thus indirectly acting as a virulence factor.

Moreover, the greater severity of the lesions induced by the *C. albicans* isolated from sheep and goats relative to the other isolates may be due to the adhesive capacity of the organism (Calderone and Braun 1991). Chaffin et al (1998), Fukazawa and Kagaya (1997) and Hostetter (1994) reported that the *C. albicans* adherence to host tissue has identified several adhesion proteins in the organism. Genes of the *C. albicans* ALS (agglutinin-like sequence) family encode proteins with features of cell surface adhesion glycoproteins (Hoyer et al 1998). Moreover, Shimizu et al (1995) reported that the *C. albicans* was the only species which could produce four enzymes (hyalurinidase, chondroituin sulphatase, proteinase and phospholipase) simultaneously from a single strain and the *C. albicans* strains which failed to produce one or more of the four enzymes seemed to be less virulent.

Finally, it could be concluded that the *Candida albicans*, isolated from goats and sheep, induced severe multiple lesions than that isolated from cattle and buffaloes.

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