

Study the pathogenesis of *Candida albicans* in animal model

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Abstract: The aim of this study was to investigate the effect Sereny test caused by *Candida albicans* in experimental induced ulcer in rabbit. Corneal ulcer was induced by superficially inoculated with 100µl of *C. albicans* containing 1.5×10^8 CFU and dropped on left eye with sterilized PBS was topically applied to right eye in 50 rabbits. These animals were divided equally into two groups. The first group with nontreated with cyclophosphamide and second group treated with cyclophosphamide (immunosuppressed). Seven days later corneas photographed and animals killed. After sacrificing the corneas of animals underwent routine histopathological examination on the day 1st, 3rd, 7th days after inoculation. Histological examination reveals that no significant changes were found in ocular tissues on the 1st day of infection in two groups, Hyphae did not penetrate into the deep layers of the cornea in any of non treated cyclophosphamide group in the contrast of second group treated with cyclophosphamide, also seen infiltration of inflammatory cells with complete destruction of retina. Experimental study of infection of *Candida albicans* in rabbits revealed a positivity for Sereny test and resulted in conjunctivitis, keratitis and finally retinitis. Histopathological changes were induced in ocular tissue associated with infiltration of polymorph nuclear cells in the infected eye by *Candida albicans*. under test.

I. Introduction

C. albicans can be acquired as the result of eye trauma and it is an occasional contaminant encountered by corneal transplant recipients (Merchant et al, 2001) and patients with chronic ocular surface disease (Tanure et al, 2000). Experimental murine keratomycosis is a reliable mammalian system for understanding the pathogenesis of human ocular infection (Wu et al, 2003).

Candida, Pathogenesis, Histopathology, Sereny test

Candida, Corneal ulcer, Rabbit.

II. Material & Methods

Experimental *Candida albicans* infection

Rabbit model

50 rabbits were designated as a young adult. The rabbits of either sex weighing 1-2 kg were used in this study. The rabbits were clinically healthy and kept in the cages at animal house, College of medicine, University of Babylon. Food and water were given freely during the adaptation period.

Sereny test

This test was performed according to the procedure described by Sereny (1957). The corneas of 50 rabbits (right eye) was superficially inoculated with 100 µl of *C. albicans* containing 1.5×10^8 CFU and dropped on left eye with sterilized PBS was topically applied to right eye.

Preconditioning with Cyclophosphamide

Cyclophosphamide was administered intramuscularly at 180 mg/kg body weight 5 days, 3 days and 1 day before the inoculation to cause immunosuppression and thereby facilitate establishment of infection.

Histological examination :

On the day 1st, 3rd, 7th, post induced corneal infection, the anterior chamber of eye was entered with a scalpel blade and the entire cornea was excised from the eye with corneal scissors.

Experimental infection in Rabbits

Determination of invasive isolates of *C. albicans* in ocular tissues was determined by using Sereny test (Sereny, 1957). In this experiment, 1.5×10^8 (CFU) was used to induce the infection in two groups of animals. The first group was not treated with Cyclophosphamide and Cyclophosphamide another treated group.

III. Results

Non – Cyclophosphamide - treated group

Histological examination

On the 1st day after infection .No significant changes were found in ocular tissues (unaffected cornea - Fig (1) and unaffected retina Fig (2)

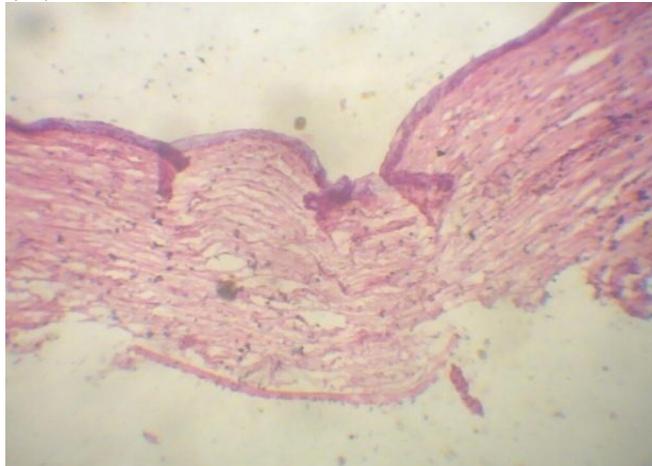


Fig (1) :Section through unaffected cornea .PAS stain . (X 100).

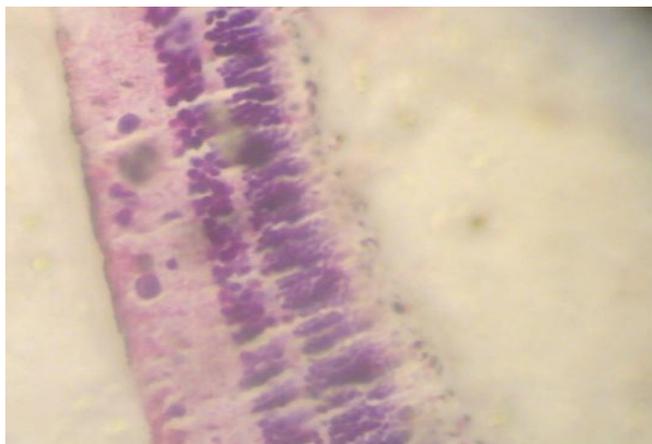


Fig (2) :Section through the retina preserved histological architecture (PAS) . X 400

On the 3rd day of infection , the first infection of *Candida* was shown in the anterior stroma with few fungal spores Fig (3) .

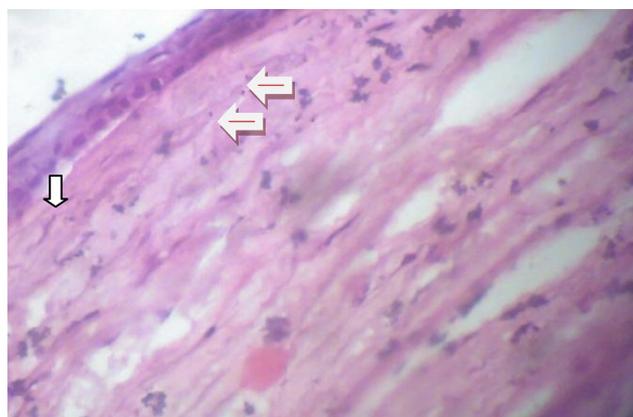


Fig (3) :Section through the cornea showing few fungal spores in the upper stroma (arrows). PAS stain. (x400)

Positive conjunctive with inflammatory cells were also seen in to the 3rd day of infection as indicated in Fig (4).

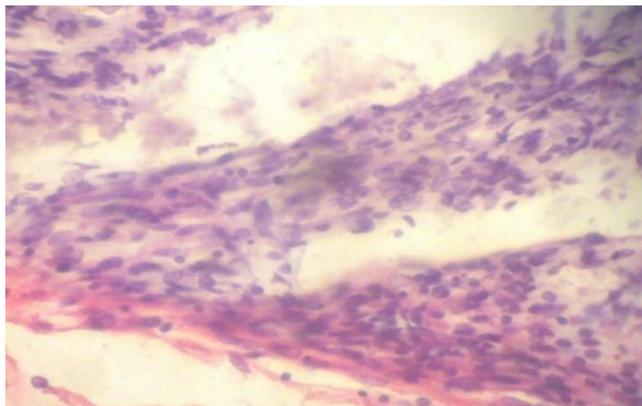
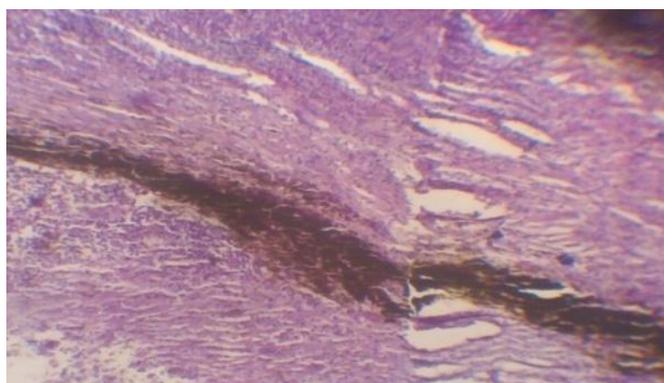


Fig (4) : Section through the conjunctiva showing inflammatory cell infiltrate .H&E , X 400 .



Cyclophosphamide – treated group .

All the rabbits were treated with cyclophosphamide before inoculation as mentioned in ch.3 .

Histological examination

Histological findings in cyclophosphamide – treated animals are shown in table (1) . On the 1st day of infection , no significant changes were found in ocular tissues as shown in non - treated cyclophosphamide – (1 st day after inoculation) .

On the 3 rd day , yeast cells had appeared in the lumen of capillaries in the ciliary and inflammatory cell infiltration of the lamina propria . Fig (5)

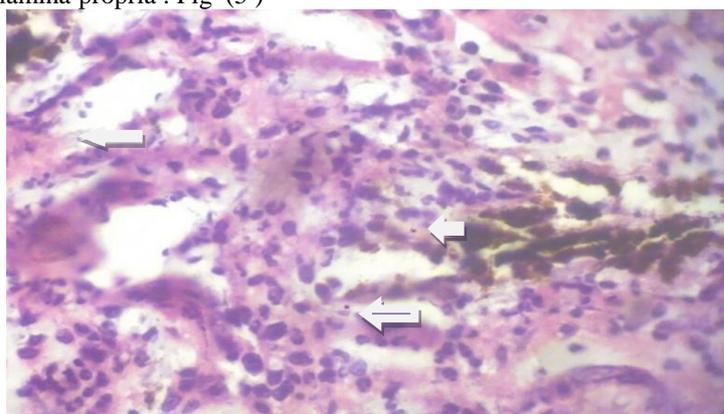


Fig (5) :Section through the ciliary body, showing inflammatory infiltration of the lamina propria, associated with fungal spores (arrows). PAS stain. (x400)

On the 7 th day , the infection of *Candida* was found in the retina , the choroidal membrane was thickened with infiltrates of inflammatory cells . The inflammatory infiltrate extended to the whole layer of the retina resulting in complete destruction of the retina Fig (6) .

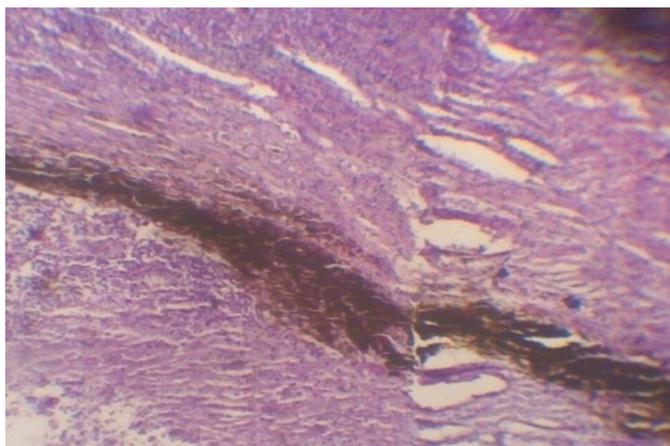


Fig (6) :Section through the retina; choroid and sclera, with inflammatory cell infiltration associated with complete destruction of the retina. H&E. (x 100)

On the 7 th day of the experiment after infection , inflammatory cells infiltration seen in which many fungi with hyphal growth were visible as shown in Fig (7)

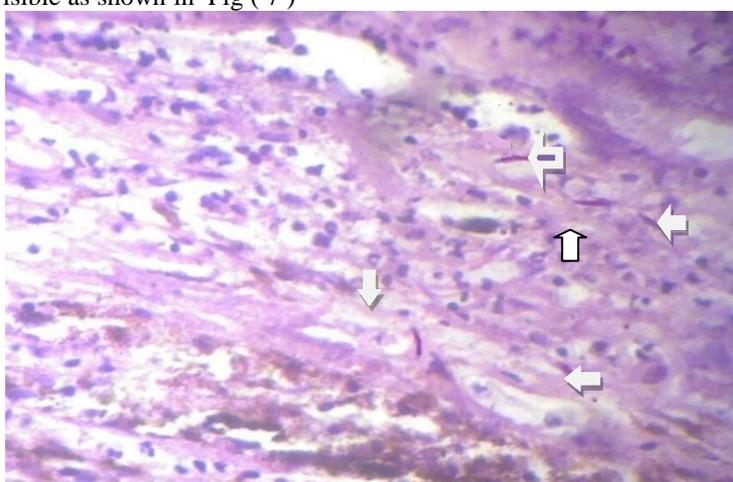


Fig (7): Section through the retina, showing inflammatory cell infiltration of the lamina propria, associated with fungal hyphae (yellow arrows) and spores (black arrows). PAS stain (x400)

Results revealed in table(1) showed histological features in non – Cyclophosphamide group , the presence of blastospores inflammatory cells infiltration compared with histological features inCyclophosphamide group , the presence of blastospores , hyphal filamentation and inflammatory cells infiltration .

Table (1): Summary histological findings in animals groups

Days finding	1		2		3	
	CNT	CT	CNT	CT	CNT	CT
Blastospores	-	-	+	+	+	+
Hyphae	-	-	-	+	-	+
Inflammatory cells	-	-	+	+	+	+

Abbreviations :

CNT: Cyclophosphamide non treated group

CT : Cyclophosphamide treated group

IV. Discussion

The present study attempted to establish positive Sereny test in an experimental model for *Candida albicans* infection using (50) rabbits in two groups .This study distinguished the invasive isolates of *C. albicans* to different hosts to induce keratitis and conjunctivitis in animal model by observed histopathological changes in eye according to Sereny test (Sereny ,1957).For histological evaluation in non Cyclophosphamide – treated group . In the first group , the animal was inoculated with 100µ of 1.5x 10⁸ CFU of *Candida albicans* suspension into the cornea according to Sereny test (1957) .

A 100 µl volume of *C.albicans* was used so that most of the inoculums would have an opportunity to attach corneal cell and non – attached cells were washed by tearing . One day 1 after inoculation with *Candida albicans* , no pathological changes were found in the cornea and retina (no corneal ulcer , the site of ulcer characterized by vascularization and inflammatory cells. (Fig 1 - Fig 2).

After 2 days, the pathological changes in the cornea were observed which represented by degeneration of epithelial cells and infiltration of inflammatory cells and fungal spores in the upper stroma , as shown in Fig(3). Also degeneration with invasion of polymorphonuclear leukocytes and desquamation of epithelial layers palpebral conjunctiva were found in Fig(4).The3rd ,7 th ulcer day was observed in the cornea Inflammatory cells infiltration was not seen in the ciliary body and iris. Also no pathological changes were observed in the retina

in the 7 th day .The invasive *Candida albicans* in penetration of cornea may be due to its ability to produce different virulence factors (De Bernardis et al ,1996 ; Gale et al , 1998 ; De Bernardis ,1999 ; Schaller et al ,1999) .Various intrinsic differences between the *Candida albicans* strains can be responsible for the observed marked difference in pathogen city in vivo . Fungal genes that control morphogenesis may be involved in study which referred to the signal transduction pathways that lead to hyphal or yeast formation in *C. albicans* demonstrated that genetic mutations in key virulence factors of the fungus have profound effect on fungal virulence (Lo et al. , 1997 ; Braun et al ., 2000) .Lo et al (1997) showed that inactivation of EFG1 lead to lock the morphologic state of the fungus in the presumed less virulent yeast form and reduces capability of hyphal formation in *C. albicans* . On the contrary , Braun et al., (2000) demonstrated that knockout deletion mutations in transcriptional repressors such as TUP1 could in fact lock the organisms in the presumed more invasive hyphal form . Also corneal epithelial cells , keratocytes and phagocytes are involved in distinguishing the pathogen and activates innate responses (Zhong et al , 2009) .For histological evaluation in Cyclophosphamide – treated group for second group , the animals were inoculated with 100µ of 1.5×10^8 CFU of *Candida albicans* suspension into the cornea according to Sereny test (1957) . the animal host was immunosuppressed by using cyclophosphomide to suppress the immunity of the host animal .

Other workers like Wu et al., (2003) observed that cyclophosphamide , a potent immunosuppressive drug that works primarily by inhibiting lymphocyte proliferation , increased the fungal keratitis , implying that lymphocytes might be involved in the pathogenesis of fungal keratitis . Some investigators argue that agents make an animal system artificial or non representative (O'Day et al ,1984; O' Day et al ,1999) . Moreover immunosuppression is an important risk factor for human *Candida* keratitis (Hemady,1995).The goals of a rabbit model of keratomycosis using an immunosuppressant are not only to mimic the extant and evolution of human disease but also to create a system with impaired the cellular and molecular mechanisms of fungal keratitis . The first retinal lesion developed in the retina appeared on the 3 rd day after inoculation , therefore the eyeballs of the rabbits obtained on the 3 th. and 7 th. day after infection ,as shown in Fig (6,7). A significantly greater inflammatory infiltration was noted in ocular tissue after 7 days compared with 1 days indicating stronger inflammatory reaction in histopathological analysis of ocular tissues with *C.albicans* isolate. These finding were in agreement with Tarabishly et al ,(2008) who noted the role of poly morphonuclear cells infiltrating the infected corneas , suggesting that the innate immunity involving various Toll – like receptors (TLRs) was the dominant host response to fungal keratitis .Other researchers demonstrated the role of macrophages during the development of fungal keratitis by histopathologic studies (Hanselmaver ,1978 ;Ishida et al ,1984) . Matsuda et al (1997) noted that the Macrophages in the sub epithelial tissue of the conjunctiva could help in non specific or specific resistance to a wide variety of microorganisms .Our findings for the two rabbit models can be summarized in table (1).The period and development of the infection were equivalent at the 3 rd day and the number of fungi was higher in the cyclophosphamide – treated group with most of them being in the hyphae associated with poly morphonuclear cells infiltration .

Wu et al (2003) observed that induced keratitis appeared on the 4 th day and in our experiment , infection was observed on the 3 rd day after inoculation of *Candida* . Both groups developed invasive corneal disease. Candidal spores invasion was limited to the stroma in non - Cyclophosphamide treated rabbits but penetrated deep stroma in most of the -Cyclophosphamide treated rabbits (cyclophosphamide) .

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