

Histological structure in immune suppression male mice brain and other natural experimentally infected with *Candida glabrata*

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Abstract

The current study included analysis the brain tissue structure after exposure to experimentally infection with *Candida glabrata* in BALB male mice which isolated from patients attending Tikrit Teaching Hospital suffering from Candidemia (38 male, 25 female) aged (82-15 years). The results indicated different histopathological changes in the brains immunosuppressed studied mice, the results represented congestion of blood vessels of the five cerebral cortex layers, as well as the foamy appearance of the matrix of molecular layer of the cerebral cortex. In contrast the non-immunosuppressed mice group included histological changes represented with rupture of the pia matter, as well as shrinking or shrinkage of the six-layer cells of the adjoining cortex of the pia matter and the emergence of vacuoles around cells and nerve fibers on a large scale. The group of mice immunosuppressed show pathological changes represented with atrophy of pyramidal cells and glial cells in all layers of the cerebral cortex, as well as the spongy-like appearance of the brain medulla, compared with the control group.

Keywords: *Candida glabrata*, brain, immunologically inhibitory, histological changes.

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Received: 17-4-2018

Accepted: 15-7-2018

التركيب النسيجي للدماغ في ذكور فئران مثبطة مناعياً وأخرى طبيعية مصابة تجريبياً بفطر

Candida glabrata

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الخلاصة

أُجري البحث لدراسة التركيب النسيجي للدماغ في ذكور فئران بعد إصابتها تجريبياً بخميرة *Candida glabrata* المعزولة من المرضى المراجعين لمستشفى تكريت التعليمي الذين يعانون من داء المبيضات الغازي Invasive Candidiasis (38 ذكر، 25 أنثى) تراوحت أعمارهم بين (15-82) سنة. وقد أظهرت النتائج وجود تغيرات نسيجية مرضية في أدمغة ذكور الفئران المصابة تجريبياً بخميرة *Candida glabrata* المثبطة مناعياً تمثلت في احتقان الأوعية الدموية للطبقات الخمس لقشرة المخ، فضلاً عن المظهر الرغوي لقلب الطبقة الجزيئية لقشرة المخ في حين أظهرت النتائج لمجموعة الفئران الطبيعية (غير المثبطة مناعياً) تغيرات نسيجية مرضية تمثلت في حدوث تمزق الغشاء، فضلاً عن إنكماش أو تقلص خلايا الطبقات الست لقشرة المخ المتاخمة للأُم الحنون، وظهور فجوات حول الخلايا والألياف العصبية وعلى نطاق واسع، أما نتائج مجموعة الفئران (المثبطة مناعياً) فقد أظهرت تغيرات نسيجية مرضية تمثلت في ضمور الخلايا الهرمية والدبقية في طبقات قشرة المخ كافة، فضلاً عن المظهر الإسفنجي للرب الدماغ مقارنة بمجموعة السيطرة.

الكلمات المفتاحية: فطر *Candida glabrata*، الدماغ، التثبيط المناعي، التغيرات النسيجية

Introduction

Candida is a Yeast or pseudo hyphae depending on the environmental condition present (1). It is part of the normal flora of human body, which is naturally present in the digestive tract, vagina, respiratory tract, and it is consider Opportunistic pathogens when the immunity body decreased that lead to Candidiasis, especially in patients with immunodeficiency such as Acquired Immunodeficiency Syndrome and malignant

tumor, diabetes mellitus and tuberculosis, leukemia and physiological conditions such as pregnancy, obesity, or the use of immunosuppressive drugs such as corticosteroids, excessive intake of antibiotics and the consequent emergence of isolates resistant to these antibiotics or an imbalance in the environmental balance of naturally occurring organisms in the body. Inhibition development and spread of candidiasis is related to environmental factors such as temperature, humidity, pH, and the type and pathogenesis of *Candida* (2, 3, 4). The severity of the disease depends on the extent of damage caused by *candidain* the target tissues and the consequences of the emergence of symptoms and signs of disease (5). The most important factors are the ability of penetrating host tissues as well as the producing of enzymes and toxins and the ability to growth in different temperatures and the possibility of transformation And the presence of cell wall (6, 7), which consists of manganese, protein and fat, and the arrangement of these components is very complex because of the morphological changes from yeast to dimorphic on the contrary, as well as the formation of endospores resistance (8). Glucan form about 70-50% of the cell wall. It also interferes with some antimicrobial agents such as Polyenes and prevents them from reaching the cellular membrane where they are located. The brain is a member of the central nervous system it is located in the skull is practically organized for almost all body activities, the brain is divided into three parts: the brain front pros encephalon, mesencephalon brain and the back brain rhomb encephalon, White matter and gray matter (9). Most white matter consists of myelin nerve fibers, as well as non-marrow fibers and progenitor cells, and the reason for their appearance in white is the abundance of myelin surrounding the nerve axes (10). The gray matter is composed of clusters of neurons and dendrites, as well as parts of the non-cortical neural cortex and progenitor cells. The reason for their appearance in gray is the lack of myelin. This substance is found in the brain in the peripheral regions Cortex, cerebellum, cerebellum and deep basal ganglia, whereas white matter is found deep in the cortex and surrounded by the basic contract (11, 12).

Material and Methods

This study was carried out in the laboratories of the College of Education for Pure Sciences/ University of Tikrit. after obtaining the isolate of the yeast *C. glabrata* from the patients attending the Tikrit Teaching Hospital which suffering from Invasive Candidiasis. The period of study was from February 2013 to August 2013, which included 63 samples 38 Male, and 25 females, their ages ranged 82-15 years, the cases were diagnosed by Specialist Physician. The samples were obtained from the blood, the experiment carried out a number of steps to obtain isolation and diagnosis. The brain Heart Infusion Agar/ Broth (Himedia) was used to isolate the yeast and increase the vitality of the isolates prior to use in the experiments (11) and prepared from brain and heart infusion 37 g, Agar 20 g, and distilled water 1000 ml. The medium was incubated at a temperature of 37°C for 72 hours, then examined by a microscopic and transplantation on the Sabouraud Dextrose Agar medium according to company instruction. The morphological characteristics of the colonies, represented by color and texture, according these characteristics ,and as well as some chemical tests the isolation was observed, Direct microscopy test was done after staining (12, 13).

- **Histological study:** Male mice of BALB/ c were used for 6 weeks with weights ranging from 22- 28 gm. They were divided into four groups with 5 mice in each group, as the following:
 - Group I: was injected with 0.1 ml of subcutaneous hydrocortisone for 4 day, and on the fifth day was injected with 0.3 ml of *C. glabrata* in the tail.
 - Group II: injected with 0.3 ml of yeast strand *C. glabrata* in the tail.
 - Group III: injected with 0.1 ml of Hydrocortisone subcutaneous for four days.
 - Group IV: injected with 0.1 ml of distilled water and considered as control group.

The mice were placed in normal conditions and monitored daily until the symptoms were appearance at 15 days after the injection with candida. After this period the symptoms were: weight loss, redness of the ear, severe sweating, hair color changed to pale yellow, As well as the emergence of one syncope case and two mice died in the third group. At the end of the experiment, the animals were dissected and samples of the brain were collected at 0.5 cm³. It was placed in Formalin at a concentration of 10% for 12 h., then washed with water for 10 minutes, followed by a series of passes with alcohol, Laminating with paraffin wax and making special molds as L-Shaped using pure paraffin wax with a melting point of 60 °C, then cut by a rotor with a thickness of 4- 5 micrometers, and flatten with alcohol by concentration 30%, and then loaded onto the glass slides, to prepare for staining, the samples were staining with hematoxylin and eosin (H and E) according to (14) and then examined under the light microscope.

Results and Discussion

Histopathological changes in the immuno suppressed mice injected with *C. glabrata* showed several pathological changes of the brain compared with the control group (Fig. 1). The histopathological changes of the brain showed that the histological sections of the mice in the first group (immunosuppressed with hydrocortisone and yeast *C. glabrata*) had a number of tissue changes in the less of nerve cells, as well as the lack of glial cells in the cortex (Fig. 2) However, the other five layers of the cerebral cortex (external granularity, external pyramidal, internal granular, inter-pyramidal, polymorphic) were uniformly shaped, with the other five layers of cerebral cortex (granular layer of the cerebral cortex) (Fig. 3). While the histopathological sections of the brain in the second group (nonimmunosuppressed) showed a number of histopathological changes in the rupture of the membrane covering the brain cortex (pia matter), while there was no change in the disease in the other six layers Other than the shrinking cortex or contractile adjacent cells adjacent to the mother of affection, so it resulted in the emergence of white area white zone around each neuron and cell support cell (Fig. 4), and contained white matter white brain large-scale large gaps great vacuoles Around cells and nerve fibers (Fig. 5). The brain tissue in the third group (injected with hydrocortisone) showed a number of histopathological changes. The cells of the six layers of the cerebral cortex were often surrounded by a white zone cavity, indicating atrophy in pyramidal cells and glial cells in these layers), While the brain core (white matter) appeared spongy like (Fig. 7)

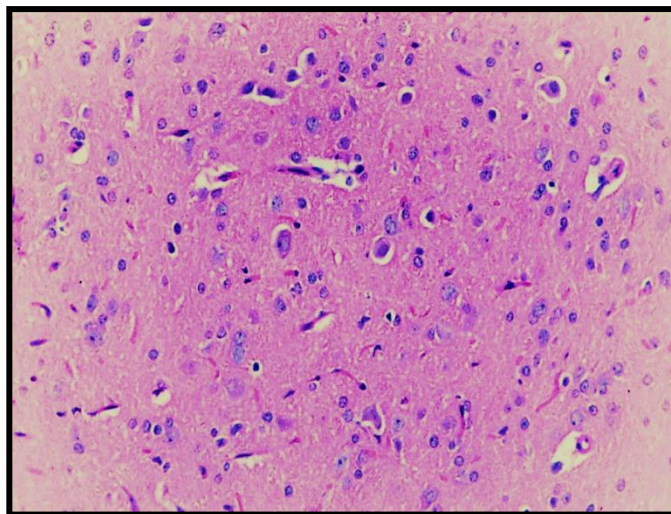


Fig. (1) A section in the brain of a mouse from the control group that detects the normal appearance of the cerebral cortex (gray or gray matter). (H &E) dye. X40

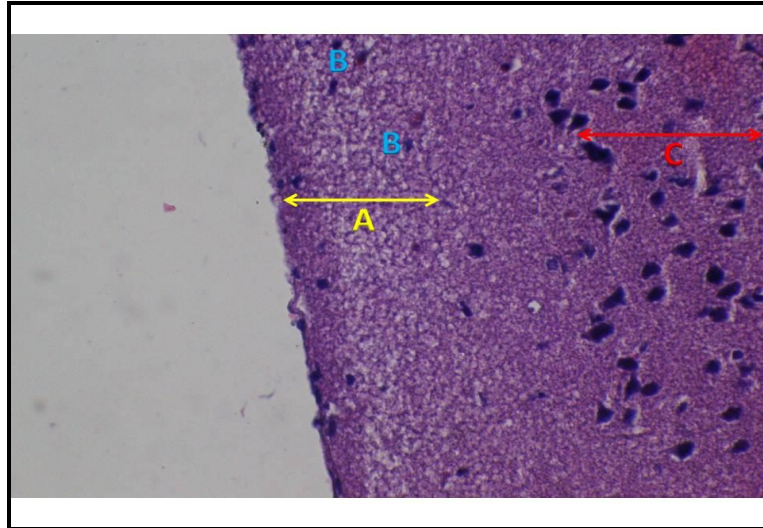


Fig. (2) A section of the brain of the first group (immunosuppressant with hydrocortisone and *C. glabrata*) in which the molecular layer of the foamy appearance (A), as well as the lack of glial cells (B), With the appearance of the external granular layer. (H & E) dye. X40.

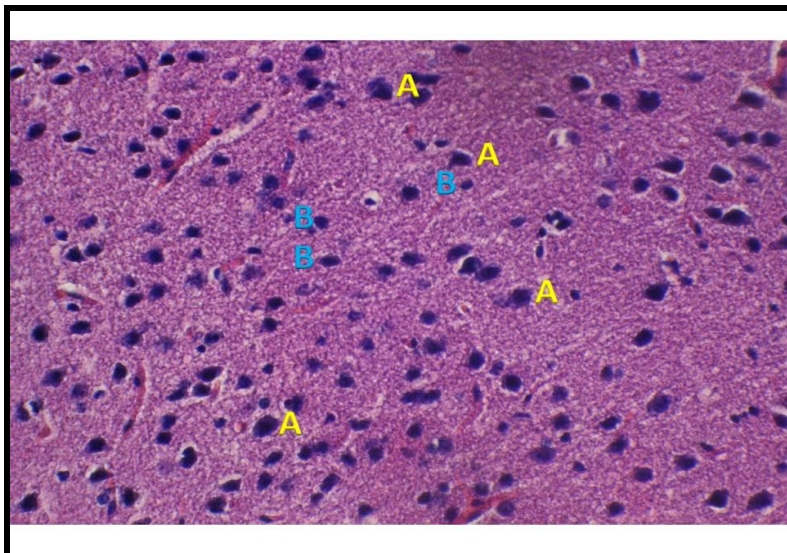


Fig. (3) A section in the brain of the first group (immunosuppressant with hydrocortisone and *c. Glabrata*) in which pyramidal cells (A), glial cells (B) of the third and fourth layers of the cerebral cortex are observed. (H & E) dye. X40

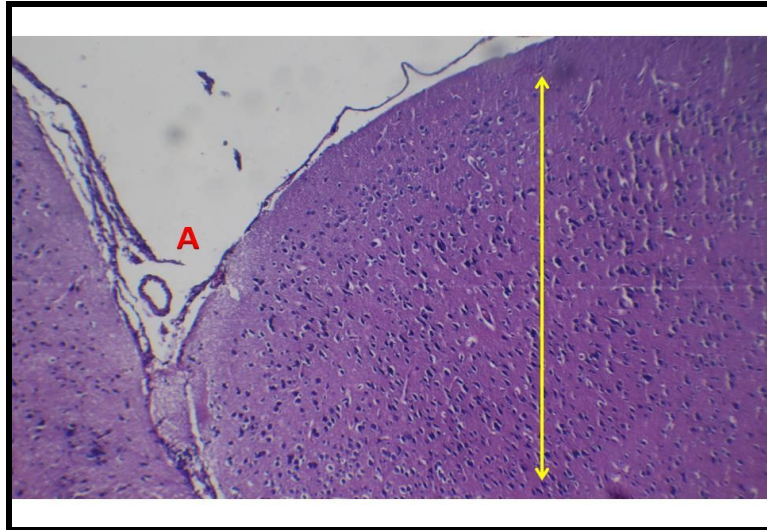


Fig. (4) A section in the brain of the second group (natural or non-immunosuppressant *C. glabrata* is observed in the rupture of the pia matter (A), the six layers of the cerebral cortex (arrow), as well as the emergence of a white area around the neurons and cells chock. (H & E) dye. X10

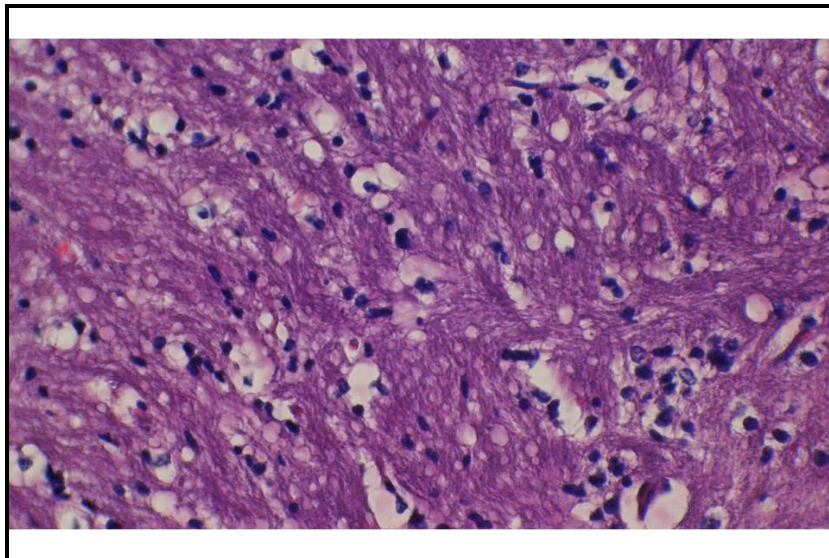


Fig. (5) A section of the brain of the second group (natural or non-inhibitory immunoglobulin *C. glabrata* in which extensive vacuoles is observed in the brain medulla around the glial cells. (H&E) dye. X40.

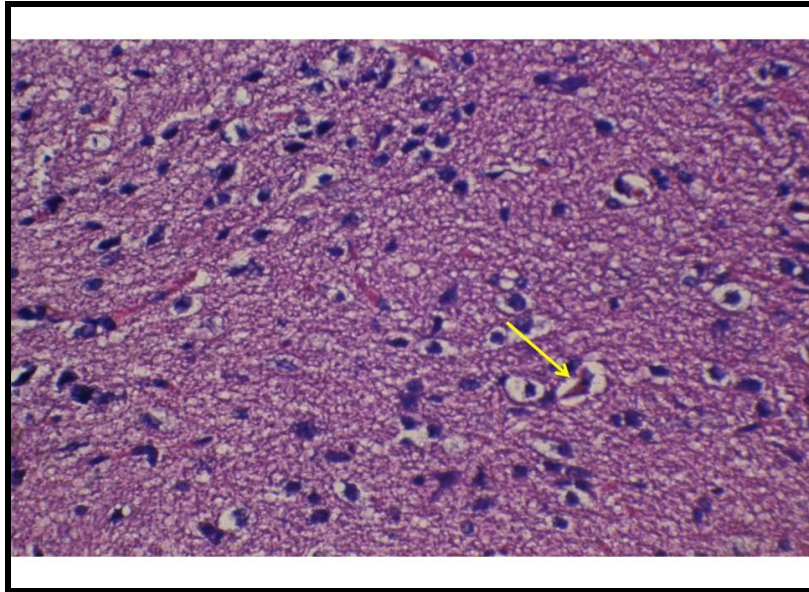


Fig. (6) A segment of the brain of the third group (injected with hydrocortisone) in which the neural cells of the cerebral cortex are observed and the zonal cavity of the external and external granular layers and polymorphisms explodes. (H & E) dye. X40.

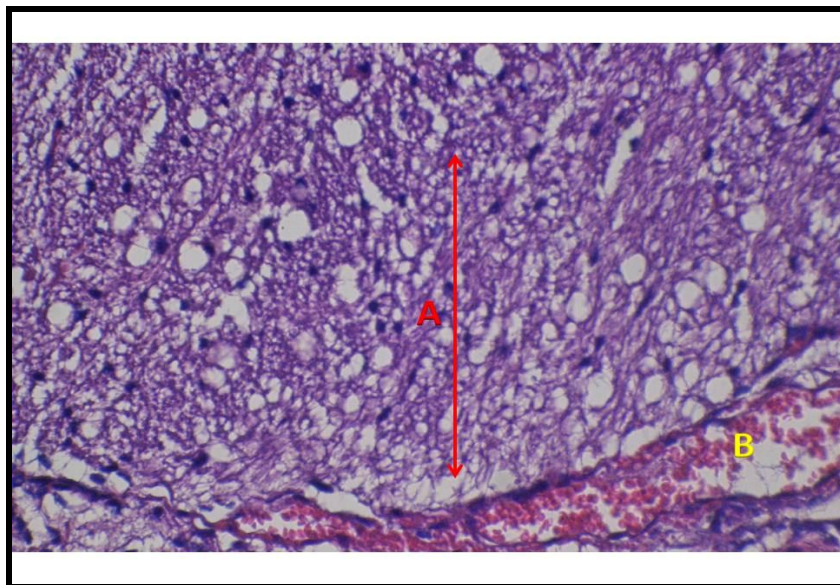


Fig. (7) A segment of the brain of the third group (injected with hydrocortisone) in which the white matter of the brain is seen as spongy like (A), as well as the cerebral artery (B), a condition similar to multiple cerebral sclerosis sclerosis (axillary sheath removal) around the axes, except for the absence of a lymph node. (H & E) dye. X40.

The ability of *Candida* to cause disease in humans or animals is a transient phenomenon (15). With the exception of a few dermal infection caused by fungi, pathogenicity is not a basic need for the survival and spread of the species (10). The two most important physiological barriers to growth of fungi within human tissues are high temperature and oxidative stress redox potential. Most fungi are mesophilic, most of which have a maximum temperature of about 35°C. Their enzymatic pathways work more efficiently in dead organic matter, which has more oxidative oxidation stress compared to oxidation stress. As well as the strong cellular defenses possessed the

human body and animal that can be inhibits the growth of the fungus. Therefore, the ability of the pathogen depends on its ability to adapt to growth and resistance to the cellular defenses of the host, when infected host cells are first in contact with the surface in mushroom cells of walls, 20% of Mannan, which is a surface antigen that can be detected in the patient's blood as well as an antimicrobial agent of the Polyenes. It is responsible for the hypersensitivity of candidiasis to these antigens. Creation of disease Constitute a means Gluing mucous membranes (16, 17). The surfaces of *C. glabrata* cells have specialized proteins in their cell walls called adhesins (18) similar to those found in *C. albicans* (19). In addition to their normal life roles they make to achieve adhesion with in Host cells including pharyngeal cells, epithelium and endothelium and thus neutralize host defenses. Check links with proteins and carbohydrates host cell membranes such as fibrinogen, fibronectin, and laminin by forming a fibril layer containing multiple sugars on their cell surfaces (20), and their ability to form a biofilm helps them to survive It is characteristic of it and can escape host defenses, as well as increase its resistance to antifungal agents and their ability to withstand competing pressure from other microbes (21). In addition, the production of hemolysin show another important factor of pathogenicity, which makes the red blood cells decomposed food as well as the solution of white blood cells, especially neutrophils active in the resistance to the incidence of *Candida* and large macrophages and the process of phagocyte is not efficient (22, 23). Its production of phospholipase contributes to damage to host cell membranes, as well as enhancing its adhesion to its cells, which increases its ferocity in invasion and destruction of mucous membranes (24, 25). Although histopathological lesions caused by *Candidain* the central nervous system are little known, anatomical studies have shown that fungal infections cause significant gray matter degradation and cortex deepest layers, where arterioles are more bent and twisted (26).

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