Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3982 - 3990
Received 08 November 2021; Accepted 15 December 2021.

Induced Changes in the Ovaries of Albino Rats by Infection with C. Albicans

Sara Reyad Ali, Saja Jamal Noaman

Department of Medical Laboratory Technology, Imam Ja'afar Al- Sadiq University, Kirkuk, Iraq

Abstract

The current study aims to determine the effects of experimental infection with *C. albicans* on the histological structure of the ovaries in pregnant female albino rats. 50 vaginal swabs for pregnant women are obtained by means of sterile cotton swabs from women who attended the gynecological consultation in the hospital and some clinics in the city. The isolates are diagnosed using the Chlamydial Spores test, as well as the diagnosis with the Vitek2 Compact system. Then, an experimental infection is induced in the reproductive system of female rats, as they are injected with a suspension of *C. albicans* yeast of 1.5 x 810 cells/ml concentration once daily until symptoms appear. The results of the microscopic examination of the ovarian tissue reveals necrosis, degeneration, lysis and desquamation of the follicular cells, congestion in the blood vessels, as well as their expansion and hemolysis , shedding of their lining, thickening of their walls, infiltration of inflammatory cells, fibrosis, severe hemorrhage in the stroma, and fibrous edema, Cytoplasmic eruption of a number of ovarian cells. Moreover, necrosis of corona radiata is found around the primary oocyte, focal pooling of inflammatory cells and an overnumber of fibroblasts.

Key words: vulvovaginal candidiasis, *C. albicans*, experimental *Candida* infection.

Introduction

Fungi are Eukaryta organisms, as there are approximately 50,000 diagnosed species that live in nature, including (80) types of molds and yeasts that cause many diseases in humans and animals alike (Jawetz et al., 2004). [1] Fungi can interact with plants, animals, and humans and establish symbiotic, commensal, latent, or pathogenic relationships (Hawksworth, 2001)[2].

Fungi are Eukaryta organisms, as there are approximately 50,000 diagnosed species that live in nature, including (80) types of molds and yeasts that cause many diseases in humans and animals alike (Jawetz et al., 2004). [1] Fungi can interact with plants, animals, and humans and establish symbiotic, commensal, latent, or pathogenic relationships (Hawksworth, 2001)[2].

The infection caused by Candida has become one of the most common and frequent fungal infections, in addition to being one of the most causative agents of serious opportunistic fungal

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3982 - 3990
Received 08 November 2021; Accepted 15 December 2021.

diseases. norvegenesis, C. kefyr, C. parapsilosismetapsilosis, C. famataNNN. ,C. guilliermondiis. And others confirmed [3], that C. albicans is the most common in a study conducted to isolate different yeasts from the vagina in women.

It has been known for a long period of time that the elderly and the young are susceptible to infection with candidiasis, and that this infection is either endogenous source by changing its behavior under certain conditions from organisms coexisting with the body to opportunistic pathogens, or by the presence of predisposing factors such as diabetes, pregnancy, Excessive use of antibiotics, steroids, and others [3].

Candida albicans yeast is characterized by being dimorphic, so it grows in an oval or filamentous yeast form, depending on environmental conditions such as temperature, pH, humidity and components of the nutrient medium, so that the yeast form can grow on acidic solid culture media containing organic sugars and nitrogenous substances that are a source of carbon and at temperatures below 35°C. As for the filamentous form, it grows on culture media that contain inorganic nitrogenous compounds, as well as culture media that contain starchy materials such as Potato Dextrose Agar and Corn Meal Agar, which have a pH of 6.5 or more [4]

Careful microscopic examination of C. albicans yeast cultured on Glucose - yeast extract - peptone medium after an incubation period of 3 days at 250 °C shows that its cells (bud spores) take an oval or spherical shape, and sometimes longitudinal, with a diameter of (4-6) micrometers, and are single or in two forms. Mycelium pairs or false mycelium, its colonies are creamy-white, smooth and convex, and have a yeasty smell when developed under aerobic conditions. In liquid media, they grow at the bottom of the tube in a sedimentary manner during an incubation period of (24-48) hours, and their old colonies are characterized by their roughness, wrinkle and edges. Irregular, in addition to its ability to form a germ tube when grown in human blood serum or egg albumin for a period of (2-3) hours at a temperature of 037 °C, and among the important and rapid diagnostic characteristics of this yeast is its ability to form large, spherical, thick-walled cells. Their diameter ranges between (8-12) micrometers, and they are peripheral or lateral at the site called spores. [5] These yeasts are also distinguished by being Gram-positive [6] in addition to the possibility of staining them with lactophenol blue to notice chlamydial spores and fungal hyphae. [7]

SymptosVulvovaginal Candidiasis

The symptoms and signs of vaginal thrush are very common, but the sensitivity of any individual sign or symptom is suboptimal for diagnosis, because its symptoms are somewhat similar to bacterial vaginosis, and in general, the symptoms that may be typical for diagnosis are: burning,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3982 - 3990
Received 08 November 2021; Accepted 15 December 2021.

itching irritation, redness, swelling, vaginal rash, and pain in the vulva and vagina, knowing that one of the most important symptoms that characterize vaginal thrush is white, thick, odorless vaginal secretions that have a cheese appearance [8] different from Infection with vaginal trichomoniasis, as shown in Figure (1).

Methods

Samples collection

50vaginal swabs for pregnant women using sterile cotton swabs are obtained from women attending the gynecological consultation in the hospital and some clinics in the city, under the supervision of a doctor. The period from the beginning of the April to September / 2020, for pregnant women group who are (17-49) years old.

Culture of samples

Vaginal swabs are transferred directly to the laboratory, and cultured on SDA medium containing the antibacterial Chloramphenicol of 10 μg/ml concentration of the medium, and the dishes are incubated in the incubator at 37º C for (24-48) hours, and the dishes that did not show growth were neglected[9].

Purification and preservation of isolates

The isolates are purified and cultured on PDA medium containing anti-Chloramphenicol and incubated at a temperature of 37º C for 48 hours, then kept in the refrigerator at a temperature of 4º C, taking into account that it is renewed monthly. As for the preservation of isolates for longer periods of time, they were cultured on slant SDA medium in a sterile small glass bottle. Then, they are incubated at 37º C for 48 hours and kept in the refrigerator at 4º C, with renewal every 3 months [10].

Preparation of yeast suspension

The SDA medium is inoculated with Candida by streaking method. The plates are incubated at 37ºC for a period of (18-24) hours. Then part of the inoculum grown on the medium is transferred by a loop to a small, sterile, hermetically sealed glass vial containing 5 ml of saline solution. Sterile physiological, mixed well, and then the number of cells was controlled to 10^8 cells/ml of the solution, by measuring the turbidity of the suspension using a turbidity meter and comparison with the standard McFarland turbidity constant solution.[11]

Identification Test

The approved traditional diagnostic methods were followed [12], as well as the use of the PhyTek device**.**

Experimental design

An experimental infection was induced in the reproductive tract of female albino rats, as they were

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3982 - 3990
Received 08 November 2021; Accepted 15 December 2021.

injected with a suspension of *C. albicans* yeast of 1.5 x 810 cells/ml concentration on the seventh day of pregnancy, once daily, and continued until the appearance of symptoms

Preparation of tissue sections

Ovarian tissue sections were prepared according to the approved standard method [10], according to the following steps: Fixation, Washing, Dehydration, Clearing, Infiltration, Embedding, Staining, Trimming and sectioning.

Results and discussion

The current study included isolating C. albicans from the vagina of pregnant and non-pregnant women in the city of Kirkuk, as 100 samples of vaginal swabs were examined, consisting of 50 swabs for pregnant women and 50 swabs for non-pregnant women, and the result was considered positive when the number of colonies was more than ten. [13] The results of laboratory culture showed that 50 vaginal swabs with a rate of 50% out of a total of 100 smears showed a positive result, with 30 swabs for pregnant women, i.e. 60%, compared to only 20 swabs for non-pregnant women, i.e. 40%, and this means that the infection of pregnant women was more From non-pregnant women, and it agrees with what was mentioned by [3].

Diagnosis of isolates using biochemical tests

Germ tube formation test

44 out of 60 isolates showed positive results for this test, when incubated ata temperature of 37˚C for a period of (2-4) hours in 0.5 ml of human blood serum, i.e. 73.33% of the total isolates, as shown in Table (2-4). This did not agree with the study of [14], as all of their isolates were positive for this test, while our results agreed with [15] in that not all isolates were positive for this test.

The germination tube is characterized by its parallel walls, and the absence of contraction in the area of its contact with the mother cell. The germinated cells of the positive isolates were observed in a shape resembling a hand mirror as described by Mackenzie (1962) [16] and shown in Figure (1).

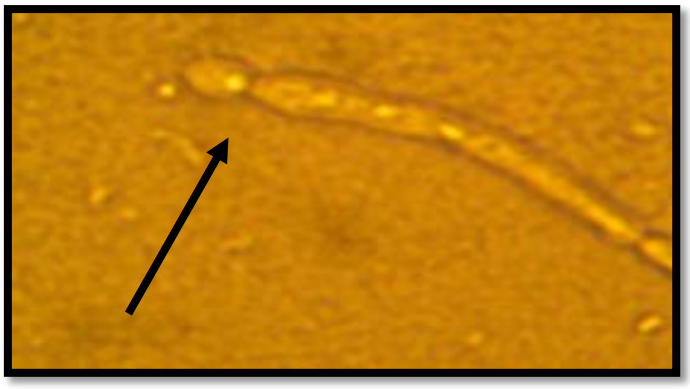

Figure (1): Formation of the germination tube (indicator) of a yeast cell growing in human serum (X40)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3982 - 3990
Received 08 November 2021; Accepted 15 December 2021.

Approximately 95% of C. albicans isolates are able to form a germination tube, and although this test is easy, fast, and relatively low cost, it has several drawbacks, including the need for the serum to be taken fresh or frozen, and the yeast vaccine must be less of 107 cells/ml, in addition to the danger of dealing with human serum with regard to the possibility of infection with some diseases, in addition to the fact that different sources of serum may give different results as well (Jan et al., 2018).[17]

4-3-3-2 Diagnosis using ChromMagar media

According to the instructions of the manufacturer of the medium and the standards specified by the researchers (14), the isolates grew well to the species level and all appeared light green, indicating that they are C. albicans as in Figure (2), and this is consistent with what was obtained. [3]

This test was carried out for the purpose of confirming the diagnosis, as Chromium Acar media is one of the most efficient and reliable media in the diagnosis of C. albicans, as well as the possibility of performing it in a short time.

Figure (2): Yeast C. albicans on chrome agar media, at a temperature of 37oC, at a age of 48 hours.

The results in both pregnant and non-pregnant groups showed that many tissue lesions occurred in the parenchyma of the target organs, and they were more in the tissues of pregnant women than in the tissues of non-pregnant women, and these results agree with Alvarez and his group (2018), [18] as the results of the microscopic examination of tissue Ovarian necrosis, degeneration, disintegration and expansion of follicular cells, congestion in blood vessels, as well as expansion and hemolysis in them, endometriosis, thickening of its walls, infiltration of inflammatory cells, fibrosis, acute hemorrhage in the stroma, fibrous edema, cytoplasmic vacuolization in a number of Ovarian cells, as well as necrosis of the corona radiata-forming granulosa cells around the primary oocyte therein, focal aggregation of inflammatory cells and an overpopulation of fibroblasts, as shown in (3), (4), (5), (6). The severity of the infection in the pregnant group, which was higher

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3982 - 3990
Received 08 November 2021; Accepted 15 December 2021.

than in the non-pregnant group, is attributed to the weaker immunity compared to the latter, and this is in agreement with [3] who mentioned that C. albicans is an opportunistic fungus that has the ability to cause severe disease in animals that suffer Immunosuppression, especially for pregnant women

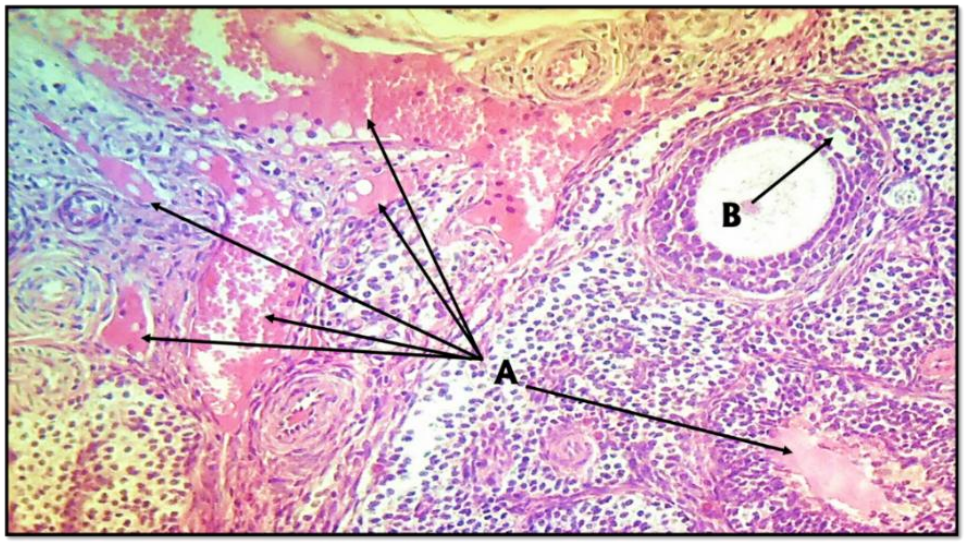

Figure 3: A microscopic image of a rat ovary from a group of pregnant women experimentally infected with C. albicans, in which congestion and severe hemolysis in a number of blood vessels (A) and necrosis of the follicular cells of a multilayered primary follicle (B) are noted. (H&E, X40.(

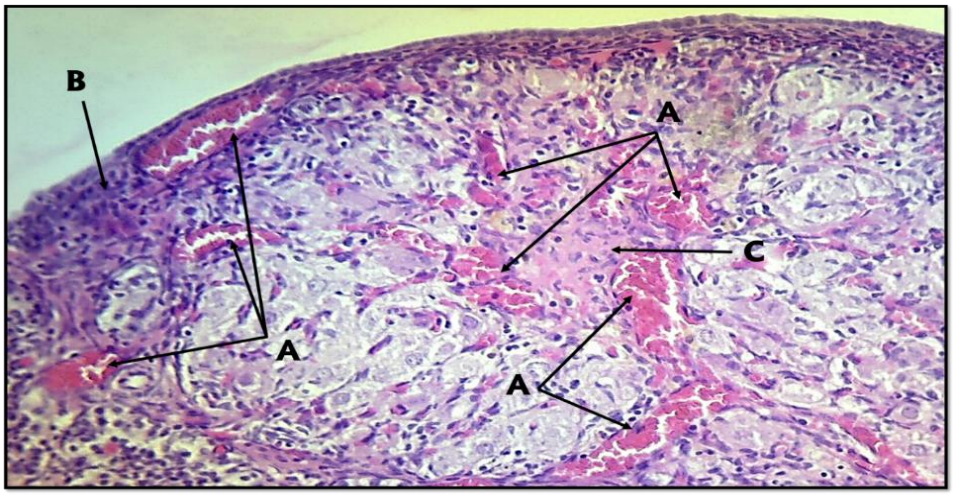

Figure (4): Micrograph of a rat ovary from a group of pregnant women experimentally infected with C. albicans, in which extensive hyperemia is observed in the cortex as well as tunica albuginea (A), dense infiltration of inflammatory cells (B), and fibrous edema between the engorged vessels (C), severe necrosis of the ovarian cells throughout the histological section, which made it lose its distinctive features. (H&E, X40.(

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3982 - 3990
Received 08 November 2021; Accepted 15 December 2021.

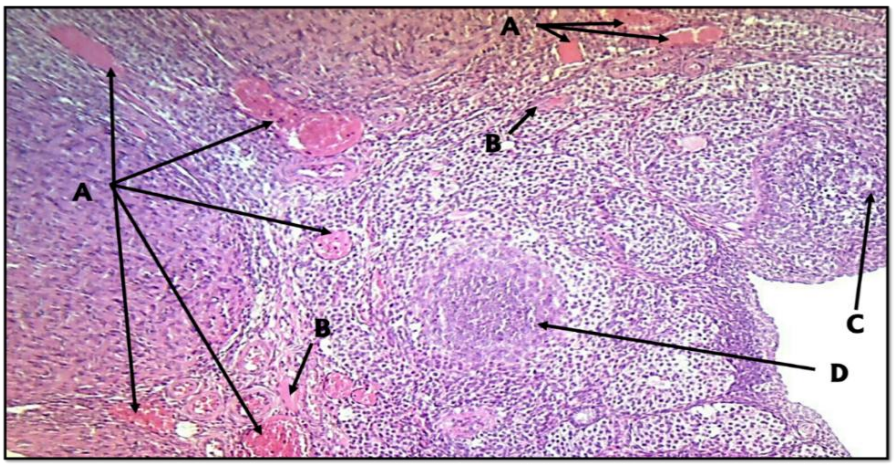

Figure 3: Micrograph of a non-pregnant rat ovary experimentally infected with C. albicans, showing congestion and extensive hemolysis in the blood vessels (A), hemorrhage in the ovarian stroma (B), acute necrosis of the follicular cells and their desquamation within the follicular cavity Multilayered primary (C), acute degeneration of another primary follicle and its appearance as a fibrous mass (D). (H&E, X40.(

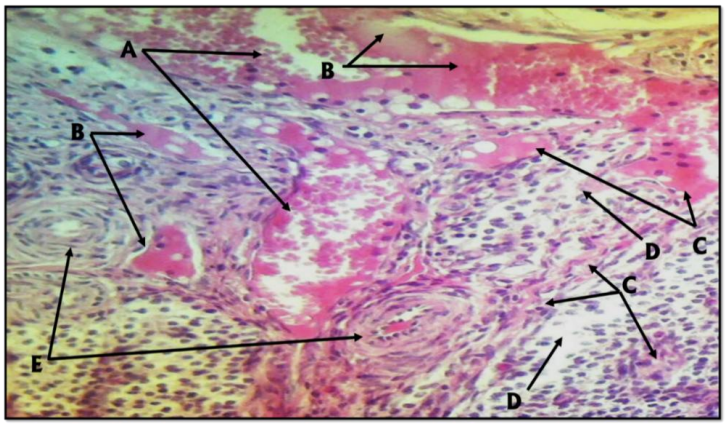

Figure 4: Micrograph of a non-pregnant rat ovary experimentally infected with C. albicans, showing severe hyperemia (A) and hematolysis (B) in a number of blood vessels, diffuse hemorrhage in the necrotic (C) ovarian stroma. Of its cells, it has lost its characteristic features (D), thickening of the walls of two blood vessels (E). (H&E, X40).

References

1. **Jawetz, E.; Melnick, J. L. and Adelberg, E. A. (2004).** MedicalMicrobiology . 20th ed. Hall international (UK), London .
2. **Hawksworth, D. L. (2001).** The magnitude of fungal diversity: the 1• 5 million species estimate revisited. Mycological research, 105(12), 1422-1432.
3- **Al-Obaidi, Maryam Tariq. (2020).** Isolation and identification of yeasts causing vaginal

thrush in women and study of the effect of some antifungals and tea tree oil extract on them. Master's Thesis, College of Education for Women - Tikrit University.

4- **Maubon, D.; Garnaud, C.; Calandra, T.; Sanglard, D. and Cornet, M. (2014).** Resistance of Candida spp. to antifungal drugs in the ICU: where are we now?. Intensive care medicine, 40(9), 1241-1255.

5- **Cletus, P. and Kurtzman, Jack, W. (1998).** The Yeasts, A Taxonomic Study,Fourth edition . New York . Oxford.

6- **Salvo, D. (2009).** Microbiology and Immunology. chapter3. *Mycology, The University of South Carolina, USA.*

7- **Webb, B. C.; Thomas, C. J.; Willcox, M. D. P.; Harty, D. W. S. and Knox, K. W. (1998).** Candida- associated denture stomatitis. Aetiology and management: A review: Part1. Factors influencing distribution of candida species in the oral cavity. *Australian dental journal*, 43(1), 45-50.

8- **Gonçalves B.; Ferreira C.; Alves C.T.; Henriques M.; Azeredo J., and Silva S. (2016)**. Vulvovaginalcandidiasis:epidemiology,microbiology and risk factors.Critical Reviews in Microbiol.

9- **Gravina, H.G.; Morán, E.G. and Zambrano, O. (2007).** Oral Candidiasis in children and adolescents with cancer. Identification of Candida spp . Med Oral Patol Oral Cir Bucal, 12(6):E419-23.

10- **Hospenthal, D. R.; Beckius, M. L.; Floyd, K. L.; Horvath, L. L. and Murray, C. K. (2006).** Presumptive identification of Candida species other than *C. albicans*, C. krusei, and C. tropicalis with the chromogenic medium CHROMagar Candida. Annals of clinical microbiology and antimicrobials, 5(1), 1-5

11- **Benke E, S., and Rogers A,L., (1980).** Medical Mycologyannual 4th Ed. Burgess Publishing Co.: Minnesota, U.S.A., 173, pp.

12- **Ferreira, I. C.; Baptista, P.; Vilas-Boas, M. and Barros, L. (2007).** Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food chemistry*, 100(4), 1511-1516.

13- **Ibrahim HI and Yehia MM. (2017).**Cervico-Vaginal Candidiasis in Married Women. Kirkuk University journal/ Scientific studies (KUJSS), 12(4):1992-0849.

14- **Al-Bajilan, A. M. N.; Bander, K. I. and Ahmed, T.(2016).** Screening Antifungal and Antibacterial Activity of Venom from Snake commonly Found in Iraq**.**

15- **Al-Jubouri, Raneen Ibrahim. (2020).** Effective content of inhibitory activity of

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3982 - 3990
Received 08 November 2021; Accepted 15 December 2021.

Ganodermaresinaceum against Candida albicans yeast associated with the genitourinary tract of women in Tikrit city. Master's Thesis/ College of Sciences/ Tikrit University.

16- **Mackenzie, D. W. R. (1962).** Serum tube identification of Candida albicans. *Journal of Clinical Pathology*, 15(6), 563-565.

17- **Jan, S.; Laba, T. L.; Essue, B. M.; Gheorghe, A.; Muhunthan, J.; Engelgau, M., ... and Atun, R. (2018).** Action to address the household economic burden of non-communicable diseases. *The Lancet*, *391*(10134), 2047-2058.

18- **Alvarez-Moreno, C. A.; Cortes, J. A. and Denning, D. W. (2018).** Burden of fungal infections in Colombia. *Journal of Fungi*, 4(2), 41.