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ISOLATE AND DIAGNOSE CANDIDA ALBICANS FROM WOMEN WITH VULVOVAGINAL CANDIDIASIS AND ESTIMATE THE LEVELS OF SOME CYTOKINES

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Abstract: Candida albicans is the opportunistic mucosal infection that causes vulvovaginal candidiasis, which affects a lot of otherwise healthy women who are of childbearing age. So, the current study aimed to isolate and diagnose C. albicans from women with Vulvovaginal candidiasis and estimate the levels of some cytokines. Vaginal and blood samples were collected from women attending Salah al-Din Teaching Hospital, Department of Obstetrics and Gynecology - Consultation Clinic, and two groups of women were selected. The first group includes 200 women suffering from infections and their ages range from 18-59 years, and a second group of healthy women (40 women) whose ages range from 17-50 years for the period from November 2023 to May 2024. 200 samples were directly inspected under a microscope; of these, 58(29.0%) were found to be positive and 142(71.0%) to be negative. On the other hand, the Human C. albicans ELISA Kit was used for the purpose of detecting positive cases and their percentage. The results found that out of 200 women suffering from vaginitis, it was found that 27.5% were positive on the test, while the negative percentage of the test was 72.5%. for cytokines, IL-10 levels in serum of patients (37.15± 5.04) demonstrated significant (P <0.05) increase in compared with healthy subjects (14.94± 1.78). the levels of IFN-γ demonstrated significant (P <0.05) increase in patients (158.74 ± 12.55) compared with healthy subjects (39.13 ± 4.25). It is concluded that using the ELISA method is the best compared to traditional methods for diagnosing C. albicans, and vulvovaginal candidiasis led to an increase in the secretion of both IL-10 and IFN-γ for the purpose of raising the immune response

Keywords: Vulvovaginal candidiasis; cytokines; INF; IL

Introduction

Vulvovaginitis, or inflammation of the vulva and vagina, is most commonly caused by a variety of underlying etiologies that impact women who are fertile. Often referred to as vaginal candidiasis or vulvovaginitis associated with candidiasis, candidiasis is caused by the polymorphic opportunistic fungus Candida albicans. Candidal vulvovaginitis is the cause of vulvovaginitis in around one-third of cases [1-2]. In 85 to 90% of cases, Candida albicans is identified and is the most prevalent pathogen. Additionally, typical is Candida spp. infection that is asymptomatic. About one-third of women had it while they have no symptoms, and during a one-year monitoring period, 70%

of women had it [3–4]. Bauters et al. discovered a 6.3% rate of clinical infection and an overall 20% colonization with Candida spp. in a research including 612 women [5]. Most women at some point in their lives have asymptomatic vaginal Candida colonization [6], and it is believed that vaginal defensive systems against Candida spp. enable their maintenance as commensals [7]. opportunistic Candida species have the ability to transform from a benign colonization to a pathogenic infection. The presence of budding yeasts and an appearance change from yeast cells to hyphae, that may cause symptomatic vulvovaginal candidiasis, are characteristics of this pathogenic infection, when anti-fungal host defenses fail to function properly or when changes in the host environment take place [8]. vulvovaginal is characterized by hyphae or pseudohyphae on a mounted wet as well as clinical signs and symptoms of vulvovaginal inflammation [6, 9]. Interleukin-17 mediated responses are crucial in reducing clinical cases of candidiasis at the oral and cutaneous mucosae, even if their role in anti-Candida immunity in the human female genital tract is still unclear [10]. Research conducted on a few mice models has revealed that IL-17 responses provided protection against murine VVC [10–11]. While Th1 cells generate IL-1β, IL-2, IL-12, tumor necrosis factor, and IFN-γ [13], Th17 cells mediate protection against C. albicans by releasing IL-17A, 17F, 21, and 22 [12]. However, both types of cells are believed to be very sensitive to HIV infection, particularly when they are activated [14–15]. Consequently, the defenses of host against infection or colonization of the vaginal region by Candida may be stimulated in the human FGT, resulting in an elevate in the production of pro-inflammatory cytokines in the female reproductive tract and an enhancement of T1/T17 cells. An elevated risk of HIV acquisition has been associated with these two occurrences [15–16]. Therefore, the current study aimed to isolate and diagnose Candida albicans from women with Candidal vulvovaginitis and estimate the levels of some cytokines.

Methods

Sample collection

Vaginal swabs

Vaginal swabs (200) were collected from women suffering from infections, which were transferred directly to the laboratory for the purpose of conducting isolation and diagnosis steps.

Blood sample

Blood samples were collected from women attending Salah al-Din Teaching Hospital, Department of Obstetrics and Gynecology - Consultation Clinic, and two groups of women were selected. The first group includes 200 women suffering from infections and their ages range from 18-59 years, and a second group of healthy women (40 women) whose ages range from 17-50 years for the period from November 2023 to May 2024. After the blood samples were taken, they were separated using a centrifuge at a speed of 4000 rpm for 15 minutes, and the serums were kept frozen until the tests required in the study were performed.

Identification

The swabs were cultured onto Sabouraud dextrose agar (Oxoid, England) in order to isolate and identify the fungus. The yeasts were grown by streaking them onto a plate and incubating them at 30 °C, per [17]. Following that, Chrom agar culture and biochemical testing were used to identify them.

Measurements

- **Human *Candida albicans* ELISA Kit**: *C. albicans* ELISA Kit (No.: SL0400Hu, SUNLONG, China) assays *C. albicans* in human serum and plasma using Sandwich-ELISA.

- **Human interleukin 10 (IL-10)**: IL-10 ELISA Kit (No.: HS-EL0261Hu, SUNLONG, China) assays IL-10 concentration in human serum and plasma using Sandwich-ELISA.
- **Human interferon gamma (IFN-γ)**: IFN-γ ELISA Kit (No.: CSL0960Hu, SUNLONG, China) assays IFN-γ concentration in human serum and plasma using Sandwich-ELISA.

Statistical analysis

The data was coded and input into a computer for statistical analysis using version 18 of the SPSS program (Statistical Package for Social Science). Each data point was arranged according to its frequency, and correlations between variables were examined using the Chi-square test. One considered a p-value of less than 0.05 to be significant [18].

Results and Discussion

Isolation of *C. albicans*

Vaginal swabs from women with vulvovaginitis were obtained for the current investigation. Using a 10% KOH solution, 200 samples were directly inspected under a microscope; of these, 58(29.0%) were found to be positive and 142(71.0%) to be negative. Table (1) and figure (1).

Table (1): Distribution of positive cultured cases

Procedures	Samples	Positive samples	
		No.	%
Direct examined by 10% KOH	200	58	29.0
Culturing Procedure	200	58	29.0

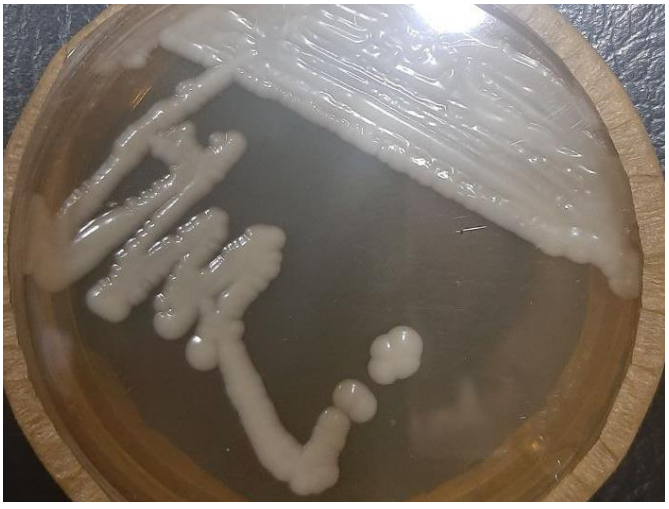

Figure (1): colonies of *C. albicans* on SDA.

On the other hand, the Human *C. albicans* ELISA Kit was used for the purpose of detecting positive cases and their percentage. The results found that out of 200 women suffering from vaginitis, it was found that 27.5% were positive on the test, while the negative percentage of the test was 72.5%, Table (2).

Table (2): Distribution of positive cultured cases

Procedures	Samples	Positive samples	
		No.	%
C. albicans ELISA	200	58	29.0

This study's findings are consistent with those of Saeed and Saadallah [19], who obtained samples from patients at Duhok Province hospitals. After these isolates were injected onto CHROM agar, the most prevalent yeast was discovered to be Candida albicans. After incubating swabs on Sabour and dextrose agar (SDA), Mohsin and Ali [20] identified every isolate both macroscopically and microscopically. Microscopic analysis was employed by Hussain et al. [21] to identify the Candida isolates. Yeasts were detected by Ozcan et al. [22] using traditional techniques like chrom agar and microscopic morphology. Of the 182 isolates, C. albicans and C. glabrata were found. The chromogenic medium's effectiveness against Candida albicans at 72 hours was 92.9%. The distinction between infection and colonization is crucial in the identification of invasive candidiasis in a laboratory because members of the genus Candida are commensal. Various types of the antibodies observed in each situation may facilitate this differentiation from a serological perspective [23–24]. The ELISA detection method is thought to be the most accurate and superior to direct methods for the purpose of detecting the presence of the infection.

IL-10 and IFN-γ

Table (3) show the levels of IL-10 and IFN-γ in patients and healthy subjects, where IL-10 levels in serum of patients (37.15± 5.04) demonstrated significant (P <0.05) increase in compared with healthy subjects (14.94± 1.78), as shown in figure (2). the levels of IFN-γ demonstrated significant (P <0.05) increase in patients (158.74 ± 12.55) compared with healthy subjects (39.13 ± 4.25), as shown in figure (3).

Table (3): IL-10 and IFN-γ **levels in groups**

Groups / Parameter	Control	Patients	P-Value
IL-10 (pg/ml)	14.94± 1.78	37.15± 5.04*	0.001
IFN-γ (pg/ml)	39.13 ± 4.25	158.74 ± 12.55*	0.001

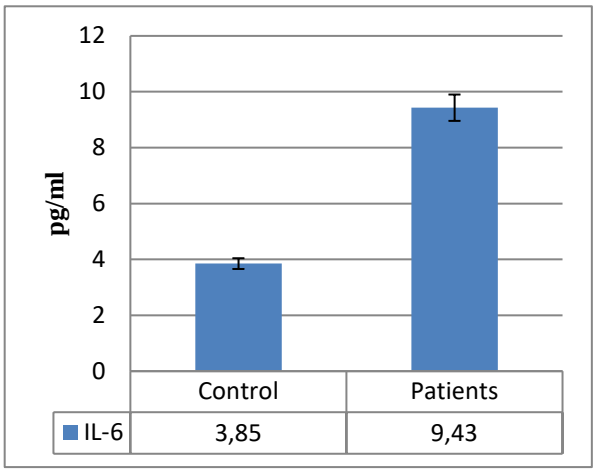

Figure (2): IL-10 levels in patients and control.

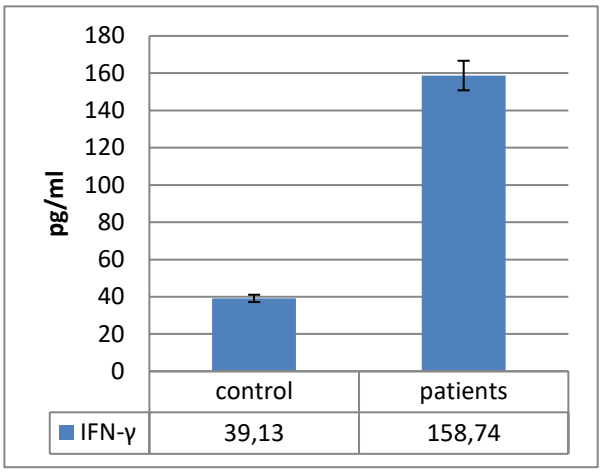

Figure (3): IFN-γ levels in patients and control.

Based on the current study's findings, there is a higher chance of vulvovaginal candidiasis development when IL-10 levels increase. According to a prior observation by Roilides, IL-10 impairs the host PMN phagocytes involved in fungal defense, hence increasing the risk of fungal infection by suppressing PMN and fungal phagocyte activity [25]. In contrast to the control group, the data demonstrated an increase in interleukin-10 levels. Ahmedi et al. [26] reported that IL-10 levels rose in cases of candidasis; IL-10 functions as a critical immunoregulator during infection with bacteria, viruses, fungi, and protozoa. Candida albicans infection of epithelial cells triggers an increase in IFN-γ production and hence initiates a systemic immune response [27]. It has been demonstrated that many mechanisms mediate IFN-γ's protective action against infections caused by Candida albicans. Early in vitro research showed that IFN-γ stimulated neutrophils' and macrophages' ability to phagocytose and destroy Candida albicans [28–29]. Increased phagocytosis and C. albicans death were observed when adult macrophages were exposed to IFN-γ [29], but no improvement was shown when cord macrophages were used in the same experimental setup [30]. These findings did not support the idea that the IFN-γ receptor is not expressed as much or that the binding of the receptor to its ligand on neonatal cells is the reason why newborn macrophages are not able to fully activate upon ingesting and killing Candida. It is noteworthy that in response to IFN-γ, neonatal cells exhibited a markedly reduced level of Stat-1 phosphorylation, indicating that there may be a negative regulation of IFN-γ receptor signaling in infants [29–30].

Conclusion

It is concluded from the current study that using the ELISA method is the best compared to traditional methods for diagnosing Candida albicans, and that vulvovaginal candidiasis led to an increase in the secretion of both IL-10 and IFN-γ for the purpose of raising the immune response

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