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Immunological study of candidiasis in women using intrauterine contraceptive devices in Erbil city

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Abstract

Among 80 samples of vaginal swabs taken from female patients at a Maternity Teaching Hospital in Erbil city for the period between May and November 2021 complained of valvovaginitis symptoms, including both intrauterine contraceptive devices (IUCD) and non-intrauterine contraceptive users. The vaginal samples from patients were culture on Sabouraud Dextrose Agar (SDA) and species identification utilizing Chrom agar. The highest incidence rate had discovered in age group of 26-35 years 17(54.84%), followed by >36 years 8(25.81%), and the lowest rates was observed <25 years which is 6(19.35%). All *Candida* isolates from positive samples were identified by using Sabouraud Dextrose Agar. The differentiation of these species is based on the colony colours produced due to reactions of species-specific enzymes with a property of chromogenic substrate. The colors of *Candida albicans* 18(58.06%) was Green shine, *C. spherica* 6(19.35%) showed light purple to purple and *C. krusei* 7 (22.58%) was Purple to Pink. Toll-like receptors TLR4 are key pattern-recognition receptors of the innate immune system in sensing *C. albicans* and the dectin-1 like receptor has shown to recognize fungal β-glucans and induce innate immune responses. As well as data indicated that IL-23 were linked with an elevated risk of VVC in Erbil.

Keywords: Candida species, CHROM agar, TLR4, Dectin-1, IL23

Introduction

` Vulvovaginal candidiasis (VVC) is the second most common cause of vaginitis after bacterial vaginosis. *Candida albicans* is the most common species; however, *non-albicans Candida* (NAC) species have increasingly been isolated (1). Clinically, VVC due to NAC is usually indistinguishable from that due to *C. albicans* (2). Vulvovaginal candidiasis means the isolation of Candida species in culture from study groups with signs, symptoms of vaginal abnormalities. Candidiasis in the vagina is commonly called a "vaginal yeast infection." Other names for this infection are "vaginal candidiasis," "vulvovaginal candidiasis," or "candidal vaginitis"(1, 3). Symptoms include a cheesy-white discharge, dysuria, dyspareunia and sexual dissatisfaction (2). There are different conventional methods for identification of yeast such as: culture on sabouraud dextrose agar (SDA) media, corn meal agar (CMA), germ tube test, carbohydrate fermentation test, and in order to accelerate detection of candida CHROM agar are used as a selective and differential media which inhibit the growth of any microorganisms except candida (4-6). The component of this media, yeast extract, chromopepton, chromogen mixture (5), chloramphenicol and agar allowing identification of candida isolates by their color and colony aspect, special colony with different colors appear on this media due to chromogenic substrates which react with enzymes produced by candida therefore, chrmogenic media has the ability to differentiate mixed candida and yeast infections (7). Several types of pattern recognition receptors (PRRs) mediate Candida recognition by innate immune cells, including Toll-like or C-type lectin receptors, but their relevance at the mucosa may well be different to the systemic environment(8). In fact, while dectin-1 is critical during systemic infections (9), its role during oropharyngeal candidiasis is minor (10, 11) and its expression can even be down regulated in oral epithelial cells when challenged with the fungus (12). IL-23 expression is strongly induced in response to C. albicans via the C-type lectin pathway (13) and is best known for regulating IL-17 production by T cells and innate lymphoid cells (14), which is of particular relevance for antifungal immunity at epithelial barriers (15). The aim of this study is to detect the role of contraceptives in causing candida infection and the relationship between Candida species infection with immune indicators

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Material and Methods

Vaginal Swabs and identification test:

The cross-sectional study was done to determine the prevalence of Candida species-caused vulvovaginal candidiasis among women which are used intrauterine contraceptives at outpatient consultation clinics for Maternity hospital in Erbil city. A total of 80 specimens from 80 apparently infected with recurrent vulvovaginal candidiasis were collected by vaginal swab. The patients groups were aged from 15-45 years. A vaginal swab from the anterior fornix has been collected from infected women after a gynecologist had examined them physically. Vaginal swab collection has been done with cotton sterile disposable swabs. The swabs have been brought right to the laboratory for 24 to 48 hours of incubation at 37°C on Sabouraud's dextrose agar. On Sabouraud´s dextrose agar the growth of yeast colonies was noticed. Most of them had a heavy growth .In sporadic cases, the growth was scanty. Scanty growth has been excluded supposing *C. albicans* as a normal flora in this case. The positive cultures were then cultured on CHROM agar media for 24 hours at 37 °C to ensure the detection of mixed infections. The CHROM agar Candida medium is a novel, differential culture medium that is employed to aid in the isolation, suppositional identification, and detection of some clinically significant yeast species and to distinguish them from other yeasts on the basis of strongly contrasted colony colors that are produced by reactions of species specific enzymes with a proprietary chromogenic substrate.

Blood Sampling:

Approximately 4 ml of peripheral blood was obtained from each subject. The samples were centrifuged to separate the serum and then enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource, San Diego, California, USA) were used to detect the TLR4, Dectin-1 and IL-23 levels in the serum according to the manufacture instruction. Results were expressed in pg/ml for TLR4, Dectin-1 and IL-23 in the serum.

Statistical Analysis:

Scatter plot graphing software (Graph Pad Prism v.7, CA, USA) is used for data analyses and the obtained results were expressed as the mean ± standard error.

Result and Discussion

In the present study, the mean age was 30.67 ± 7.861 years, and the highest rates of incidence was expressed in the age groups of 26-35 years 17(54.84%), followed by >36 years 8(25.81%), and the lowest rates was observed in less than <25 years which was 6(19.35%). Table 1 illustrates the age distribution of women's who were initially diagnosed with VVC.

Table 1: Incidence of vulvovaginal candidiasis in women different age group with intrauterine Device

Pathogenic Groups	Isolation of Candida spp.	Age group					
		<25	Percentage (%)	26-35	Percentage (%)	>36	Percentage (%)
Infective with IUC	15	3	9.68	10	32.26	2	6.45
Infective without IUC	8	1	3.23	5	16.13	2	6.45
Healthy with IUC	5	0	0.00	2	6.45	3	9.68
Healthy without IUC	3	2	6.45	0	0.00	1	3.23
Total	31	6	19.35	17	54.84	8	25.81

P=0.1170

The positive colonies on SDA were re-cultured on HarlequinTM Candida Chromogenic plate (Acumedia Lab, Neogen, U.K.) and incubated at 37°C for two days. This medium is usually used for identification of species of *Candida*. The differentiation of these species is based on the colony colours

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produced due to reactions of species-specific enzymes with a property of chromogenic substrate (Table 2). According to the Company guidelines, the colours of colonies are as follows: *Candida albicans* (Green shine), *C. spherica* (light purple to purple) and *C. krusei* (Purple to Pink) (Academia lab HAL019 UK).

Table 2: CHROM agar are used for identification of Candida species

Candida species	Colony characteristic on CHRomogenic agar	No. of isolates total (31)	Percentage (%)
C. Albican	Light smooth green colonies	18	58.06
C. spherica	light purple to purple	6	19.35
C. krusei	Purple to Pink	7	22.58

To determine the role of TLR4 in the host defense against *Candida species*, we take the serum from intrauterine contraceptive women with Candida infection and compared with their not using contraceptives women. The results showed in table 4.4, that there is a production of TLR4 in women with intrauterine contraceptive infected with *C. species* (3.92±0.123) as compared with healthy women without IUC (2.16±0.09).

Table 3: TLR4, Dectin-1 and IL-23 response to Candida species

Immuno markers (pg/Ml)	Patients groups			
	Infected with IUC (Mean±standard error)	Infected without IUC (Mean±standard error)	Healthy women with IUC (Mean± standard error)	Healthy women without IUC (Mean± standard error)
TLR4	3.92±0.123	3.41±0.137	2.53±0.09	2.16±0.09
Dectin-1	9.72± 1.71	0.91±2.32	0.39±0.71	0.20±0.32
IL23	730±65.6	536±41.2	381± 28.2	259±32.1

Discussion

In the present study, the mean age was 30.67 ± 7.861 years, and the highest rates of incidence was expressed in the age groups of 26-35 years 17(54.84%), followed by >36 years 8(25.81%), and the lowest rates was observed in less than <25 years which was 6(19.35%). Table 1 illustrates the age distribution of women's who were initially diagnosed with VVC. The result agreed with (16) which revealed that, the age group of 20-29 year-olds had the highest frequency of VVC (48.5%). No significant correlation was found between age and occurrence of VVC (P = 0.137). Furthermore, (17) in United Arab Emirate, revealed that, out of 224 vaginal swabs were taken, 31.6% of women have vulvovaginal candidiasis and the age groups between 26-30 were found to have the highest percentage of positive cases (39%) and the lowest rates was observed in age groups above 40 which was (14%), but the result disagreed with (18) in Iraq which revealed that, the prevalence rate of candidiasis between specimens in the age group of 23-26 was high (37.3 %) with contraceptive users. As well as, in France (19) illistrated that, the highest rates of VVC were revealed in the age groups of 18-25years which was (7.1%), and the lowest frequency was found in age group 31-35 years which was (6.9%). Sexually active younger women who are less able to protect against Candida species in their vaginal environment (20, 21). By this way, the highest rate of vulvovaginal candidiasis was found in age group (26-35) years which were 58.84% in intrauterine contraceptive device and the lowest rate was observed in age group less than 25 years which was 19.35%. Also, in our observation, after 36 years of age, the prevalence rate of candidiasis was declined gradually. Foreign objects with long-term insertions into the uterine mucosa are known as intrauterine contraceptive devices, and the device's tail serves as a repository for yeast cells (22).

According to the Company guidelines, the colours of colonies are as follows: *Candida albicans* (Green shine), *C. spherica* (light purple to purple) and *C. krusei* (Purple to Pink) (Academia lab HAL019 UK). These agreed with (23) which revealed that, the medium supported the growth of all the clinical isolates and reference strains. A wide variety of colony colours was seen, some of which were species-

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specific (*C. albicans* appear (green), *C. krusei* (Pink), *C. tropicalis* (Dark blue) and *C. glabrata* appear (light to dark brown). The white ⁄ opaque background of CCA seemed to allow good discrimination among colonies with relatively similar hues. Furthermore, (5) revealed that, on CHROM agar media, the appearance of colonies of Candida spherical were Lavender coloured. These findings indicate that CCA allows presumptive identification of *C. albicans*, *C. tropicalis* and *C. krusei* with high sensitivity and specificity, particularly after incubation at 37C for 48 h. The results essentially confirmed data reported previously concerning the usefulness of CCA for the identification of these three species (24). Based on the available data, it therefore seems that CCA can be used to reliably differentiate *C. albicans*, *C. tropicalis* and *C. krusei*, with a discriminatory power comparable to that of CHROMagar Candida (25).

CHROMagar *Candida* is a selective and differential medium which is widely used for the rapid identification and differentiation of *Candida species* from the clinical specimen. Its superiority to SDA lies on its ability to selectively inhibit bacterial growth (6). It facilitates the detection and identification of *Candida species* from mixed culture and provides result in 24-48 hours and has an advantage of being technically simple, rapid and cost effective as compared to the conventional methods (26). In our study, we identified different *Candida species* based on colour exhibited. (7) in their study described that CHROM agar Candida can readily be used in isolation of yeast from clinical specimen and reported that use of this medium allows rapid identification of clinically important Candida species.

To determine the role of TLR4 in the host defense against *Candida species*, we take the serum from intrauterine contraceptive women with Candida infection and compared with their not using contraceptives women. This agreed with (27), In the first experimental model of disseminated *C. albicans* infection, reported an increased susceptibility of TLR4-defective mice to disseminated candidiasis due to a decreased induction of KC and MIP2. The human TLR4 polymorphisms Asp299Gly and Thr399Ile led to a higher susceptibility for Candida blood stream infections (28). In addition, stimulation of DCs by *C. albicans* induces TLR4-dependent production of cytokines, such as IFNγ and IL-12, resulting in a Th1-mediated cellular response (29). Although the recognition of *C. albicans* by TLR4 has been shown in vitro and in vivo, differences between various candida strains may account for reports showing that TLR4 plays a minor role in candidiasis (30). Furthermore, TLR4 polymorphisms were shown to contribute to a higher risk of systemic Candida infection. On the other hand, Dectin-1, a pattern recognition receptor involved in antifungal immunity (Table 3). Dectin-1 recognition of β-glucans can result in the induction of a number of cellular responses, including ligand uptake by phagocytosis and endocytosis, dendritic cell maturation, the respiratory burst, the production of arachidonic acid metabolites, and the induction of numerous cytokines, including tumor necrosis factor (TNF), IL-10, IL-2, IL-23, and IL-6, as well as chemokines like CXCL2 (31). While Dectin-1 is mainly recognized as an important mediator of innate anti-fungal responses, it has also been shown to regulate T cell responses (32). A higher level of IL-23 was observed in intrauterine contraceptive women (730±65.6) than those healthy women without IUC (259±32.1). In this study, the results are similar to (33) that change of IL-12 previously reported with vaginal candidiasis, suggesting that IL-23 also plays a protective role in mice with vaginal Candidiasis. The delayed secretion time of IL-23 as compared with 1L-12 suggested that these two cytokines may play their protective role via different pathway (34). IL-12 has been identified as a soluble factor that can stimulate natural killer cells to produce IFN-y, and reversely activate NK cells. IL-23 has limited effect in producing IFN-y. Instead, IL-23 can stimulate CD4+ T cells to produce IL-17. IL-17 can induce these T cells to produce pro-inflammatory cytokines, which contribute to autoimmunity or protective responses during infection (35). But it is unclear that whether this is a consequence of the production of IL-t7 alone, as stimulated by II.-23, or the action of IL-17 together with other cytokines, such as IL-6 and TNF, that are known to promote resistance to this organism (33).

Conclusion:

A strong association have been found between the prevalence of vaginal candidiasis and its Candida species and the use of contraceptives. This study demonstrates the effectiveness of CHROM agar Candida medium. This media has the capacity to quickly identify Candida species, and it can be utilized as a helpful supplementary media in the clinical laboratory to find Candida species. Toll-like receptors TLR4 are key pattern-recognition receptors of the innate immune system in sensing *C. albicans* and the dectin-1 like

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receptor has shown to recognize fungal β-glucans and induce innate immune responses. As well as data indicated that IL-23 were linked with an elevated risk of VVC in Erbil.

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