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*Research Article*

# Detection of anti-mannan antibodies and TLR9 as alternative methods for diagnosis of candidiasis in immunocompromised patients with vulvovaginitis

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**Abstract**
Vulvovaginal candidiasis is considered one of the most common women's infections. The main cause of this disease is *Candida albicans,* which have many virulence factors such as germ tube formation, chlamydospore, and many hydrolytic enzymes. The current study aims to use anti-mannan antibodies and TLR9 to diagnose candidiasis in immuno-compromised patients with vulvovaginitis. A total of two hundred samples were obtained from patients attending Kirkuk Tumor Center, Kirkuk, Iraq, of which 100 were vaginal swabs from immunocompromised patients aged between (18-<40 years) from the period November 2022 to March 2023. The swab samples were transported with brain-hart infusion broth (Himedia-India). Furthermore, the other 100 samples were blood samples that were separated to use the serum for the detection of TLR9 and anti-mannan antibodies. The findings indicated that *Candida spp*. was present in 83% of the cultured samples. A notable rise in TLR9 was observed in serum samples that tested positive for candida spp. The results of the sensitivity and specification of IgM detection using the ELISA test showed 100% and 89.4%, respectively. The same test for the detection of IgG showed 92.4% and 51.1%. The positive and negative agreement between the ELISA test for detecting IgM and the ELISA test for detecting IgG is 81.4% and 44.1%, respectively. The findings suggest that the use of anti-mannan antibodies to diagnose candidiasis should be considered and given more importance for identification purposes.

**Keywords:** Anti-mannan Antibodies, IgG, Immunoglobulin, Toll-like receptor

## INTRODUCTION

It is established that fungal infections of the vagina and vulva are the second most common cause of infection after bacterial vaginosis (Małgorzata *et al.,* 2023). Vulvovaginal candidiasis (VVC) affects almost three-quarters of women during their period of reproduction, and half of them experience two or more episodes (Blostein *et al.,* 2017). *Candida albicans* is considered to be the most frequently isolated pathogen causing such infections in 85 to 90 % of the whole infections (Gómez-Gaviria *et al*., 2023). However, asymptomatic *Candida spp.* infestation is also prevalent. It is discovered in roughly one-third of women who have no symptoms and was found in 70% of women over a one-year period (Tsega and Feleke, 2019). Bauters *et al.* also discovered that *Candida spp.* colonization forms 20% of overall infections and a 6.3% risk of clinical infections in samples obtained from 612 women (Bauters *et al*., 2002). Furthermore, *Candida spp.* colonization has been identified in 10 to 20% of females having conization for cervical intraepithelial neoplasia (Pathakumaria *et al.,* 2020).
Candida species are known to have a wide natural in-

habitance range and can be found in humans, wild and domesticated animals, as well as a variety of places, including hospitals (Gonçalves *et al*., 2016). These yeasts are common among human flora and can colonize the mucosal surfaces of the vaginal, urinary, respiratory, and digestive systems. In addition, they are found in the dental cavities, between fingers, on the scalp, and on the epidermis (Dignani *et al*., 2009). *Candida species* can turn from being commensal microbes or symptomless colonization into infectious pathogens. As a result, these species are classified as opportunistic since they can shift from innocuous to harmful depending on the host environment. Although *Candida* infections are mostly superficial and significant, systemic infections can arise in highly immunocompromised patients (Ascioglu *et al.*, 2002; Branco *et al*., 2023; McCarty *et al*., 2021; Pappas *et al*., 2018).

Numerous virulence factors that allow for adherence and invasion to the target cell surface, biomass formation, the yeast-to-hyphae transition, the production of tissue-damaging hydrolytic enzymes (such as hemolysins, phospholipases, and proteases), and immune cell evasion all contribute to Candida's pathogenicity (Neji *et al.*, 2017). Virulence variables can vary depending on the infecting species, host response, infection type, infection site, and geographic origin. In the past, we found that, according to multilocus sequence typing, more than half of the genotyped isolates causing bloodstream infections (BSIs) in patients at our hospital had new genotypes (MLST) (Boonsilp *et al.*, 2021).

Candidiasis, the infection caused by Candida spp., can be diagnosed by direct examination, culture technique, PCR test, and immunoglobulin detection against Candida mannan and candida arabinitol (Shukla *et al*., 2021). Mannan is the primary antigen of the candida cell wall, accounting for more than 7% of its dry weight. Antibodies against Mannan can be identified using a variety of serological tests, most notably immunosorbent assays using enzymes (Mikulska *et al.*, 2010). Initial antifungal immunity began with recognising candida antigens such as phospholipomannan by TLR2 and O-linked mannan by TLR4. TLR9 recognizes candida cell wall components, particularly *Candida albicans* (García-Carnero *et al*., 2020, Żelechowska *et al*., 2021 , (Francisco J. et al.,2021). Therefore, the current study aimed to use anti-mannan antibodies and TLR9 to detect candidiasis in vulvovaginitis patients. So, the present study aimed to use anti-mannan antibodies and TLR9 to diagnose candidiasis in immuno-compromised patients with vulvovaginitis.

## MATERIALS AND METHODS

### Sample collection

A total of 200 vaginal swab samples were collected from patients aged 18 to 40 years who visited Kirkuk Oncology Center, Kikrkuk City, Iraq, between November 2022 and March 2023. The collection process began with disinfecting the vagina with saline solution, taking the swabs, and depositing them in sterile glass vials for later. These swabs were cultured on Sabouraud Dextrose Agar in sterile glasses or plastic Petri dishes, and then they were incubated at 37 ºC for 24-72 h after having been screened for the presence of fungi(Abed *et al.*,2022). The samples obtained after culture were as follows:

Vaginal swabs: 100 vaginal swabs were collected from Immunocompromised (pregnant women, diabetic women, women treated with anti-inflammatory drugs, etc.) patients with vulvovaginitis, then the swabs were transported with Brain Heart Infusion Broth (Himedia-India).

Blood sample: 100 blood samples were collected and centrifuged to separate the serum used to detect TLR9 and anti-mannan antibodies(Abdulateef S.M *et al.*, 2024).

### Direct examination

The samples were placed on a microscopic slide, and a few drops of 10% KOH were added (a cover slip was used, and the mixture was warmed up over a gentle heat slightly below the boiling point). To detect the fungi and their septate hypha, the slide was examined with low power (40x) and high power (100x) objectives (Emmons *et al*., 1977).

### Sample culturing

After growing on Sabouraud dextrose agar (SDA) with 0.04 mg/mL chloramphenicol to prevent bacterial growth, the samples were incubated for 10 days at 28° C and 37°C under continuous observation (Midgley *et al*., 2004).

### Laboratory characterization of fungal Isolates

The phenotypic characterization of the colonies depended on their morphological characteristics, such as shape(Adil *et al.*,2019), color, size, and texture. This was performed by placing a part of a fungal colony onto a glass slide with a sterile vector. A drop of cotton blue lactophenol stain was added, and the slide was then examined under a microscope to see the properties of the mycelia (Kwon-Chung *et al*., 1992).

### Biochemical tests

**TLR9:** It was detected in the samples' sera by the use of Human Toll-Like Receptor 9 (TLR-9) ELISA Kit-MYBIOSOURCE-USA and according to the manufacturer's instructions (Al-Shmgani *et al.*,2019).

**Anti-mannan antibodies (IgM and IgG):** They were detected using the kit provided by Dynamiker Biotechnology, Tianjin, China, and according to the manufacturer's instructions.

**Table 1.** Anti-mannan IgM, IgG and TLR9 levels of anti-mannan antibodies in the serum of patients with vulvovaginitis

| ***Candida* spp. culture** | | **Anti-mannan IgM antibodies** | | **TLR9 (ng/ml)** |
|---|---|---|---|---|
| **Cases** | **Number** | **Positive cases** | **Negative cases** | |
| Positive case | 83 | 81 (97.5%) | 2 (2.4%) | 17.3±2.31 |
| Negative case | 17 | 0 (0%) | 17(100%) | 4.17±1.19 |
| Totals | 100 | 81(81%) | 19(19%) | 100(100%) |

**Statistical analysis**

The findings of the current study were statistically analyzed using SPSS version 23. Arithmetic means were chosen using Duncan's multiple range test at the probability level (p 0.01) to reveal the significant differences between groups (Al-Halbosiy *et al.*,2018).

**Ethical approval**

This study was approved by the Human Ethics Committee of Kirkuk Oncology Center. The patients' consent was obtained after they were informed about the investigational nature of the study and the results reported. The patients were also introduced to the research objectives.

## RESULTS

The culture results showed that *Candida spp.* was detected at a rate of 83% (83 out of 100). As shown in Figure 1, the colony appeared white, creamy, and viscous.

The levels of TLR9 (17.3±2.31) in a positive group for *Candida spp.* show high significant (P≤0.05) elevated compared with a negative group for *Candida spp.* group (4.17±1.19), as shown in Table 1.

The ELISA sensitivity and specificity tests to detect IgM 81 (97.5%) (Table 1). The same tests conducted to detect IgG showed 61(73.4%) out of 83 positives for *Candida spp.* culture (Table 2) ( Salih *et al.*, 2022) . There was an 81(81%) positive correlation between the ELISA tests for IgM and 66(66%) for IgG. There was a 19% negative correlation between the ELISA test for IgM detection and the ELISA test 43% for IgG detection (Abdullah *et al.*,2019) .

## DISCUSSION

The results of the current study disagree with those of the isolation and differ with those of the rate of detection, which is obtained by Yadav and Prakash (2016), who reported that the prevalence of Vulvovaginal candidiasis was 35% of a total of 157 women; the results of the current study were also higher than the study of Álvarez Botas et al. ( 2021), which indicated that among 134 samples, 31.34% were culture positive for *Candida spp*. This is due to the differences between the locations of the current study, the compared studies, and the different groups of the examined patients. Table 1 shows a significant increase in the levels of TLR9 in the serum samples, which gave positive results for *Candida* spp culture (Awad *et al.*,2020 , Dheeb et al.,2019) . The results TLR9 levels in the serum of patients agree with Matsumoto *et al.* (2021) and Mellinghoff *et al*. (2022). The increase in TLR9 levels might be due to the ability of TLR9 to detect the *Candida spp.* DNA (unmethylated CpG sequences) stimulates the dendritic cells to produce cytokines such as IL-4 and IL -10 (Yadav *et al*.2016; Ma *et al*. 2021). *In vivo* and *in vitro* studies have implicated TLR2, TLR ( Mahmood *et al.*, 2019) and TLR9 in innate and adaptive immunity to *C. albicans* and *A. fumigatus*. However, the precise roles of individual TLRs in orchestrating the complex inflammatory pattern that follows *in vivo* fungal infection and the cell biological processes that underlie TLR-mediated host-pathogen interactions remain poorly understood (Netea *et al.*, 2006; Romani 2011). Both TLR have been shown to play an important role in recognising fungi ( Abed *et al.*, 2019) either alone or in cooperation with other pattern recognition receptors, and they enhance innate effector functions (Romani

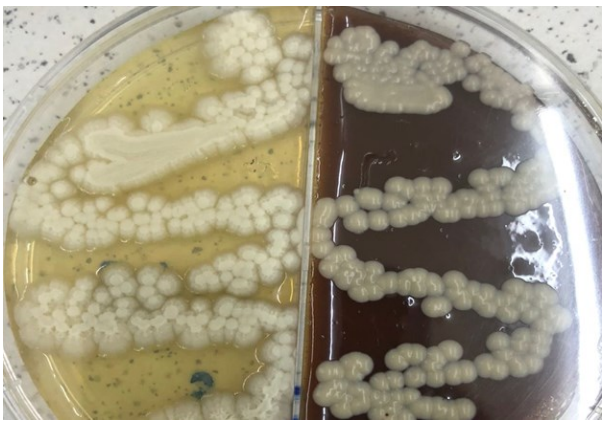

**Fig. 1.** *Candida spp. colony on Sabouraud dextrose agar and Chocolate agar*

**Table 2.** Anti-mannan IgG antibodies in the serum of patients

| **Candida spp. culture** | | **Anti-mannan IgG antibodies** | |
|---|---|---|---|
| Cases | Number | Positive cases | Negative cases |
| Positive case | 83 | 61(73.4%) | 22 (26.5%) |
| Negative case | 17 | 5(29.4%) | 12 (70.5%) |
| Totals | 100 | 66 (66%) | 43(43%) |

2011).

Table 2 shows that most of the positive cases *for Candida spp* culturing test were positive for the ELISA detection test for Anti-mannan antibodies. These results agree with those of Mikulska *et al. (*2010), Altintop *et al.* (2021), and Nakayama *et al.,* (2022), who indicated that there is a relationship between positive cases of *Candida spp* culture test, most of which were positive for the ELISA detection test for Anti-mannan antibodies. The positive cases to the culture test and negative to the ELISA test for the detection of IgG might be due to the time gap between the infection and the time conduction of the study. After 7-10 days of the infection, the first immunoglobulin type raise was in IgM, which switches the IgG production, Allaw *et al., 2021.* Table (2) also shows negative cases for *Candida Spp* isolation test and positive cases for the ELISA detection test for IgG ( Hussain *et al.*, 2018 , Nasser et al. 2018). This might be due to a previous infection that the patients were cured of; therefore, the causative agents disappeared,( Paulovičová *et al*.,2022) but the antibody titer remains high (Cao *et al.*2021). Table 2 also shows that the ELISA test for IgM detection had a higher sensitivity than ELISA detection test for IgG. These results agree with those of Shukla *et al.* (2012).

According to Parra-Sánchez *et al*. (2017), the *C. albicans* germ-tube specific IgG antibody assay has a sensitivity range of 61.1% to 85.7% and a specificity range of 75.8% to 80.3%. According to a study by Mattsby-Baltzer *et al*. (2015), IgG2 anti-phosphopeptidomannan antibodies are another biomarker for invasive candidiasis with 88% sensitivity and 94% specificity. However, ( H.S. *et al.*,2022, Dheeb *et al.*,2022) no IgG2 antibody response was seen in *C. parapsilosis* or *C. albicans* infections. When the ideal positive threshold was 70 pg/mL, White *et al*. 2017 reported that the sensitivity and specificity of a (1-3)--D-Glucan (D-BDG) assay were 90.7% and 73.4% for the detection of invasive fungal illness, respectively. Semi-nested PCR (snPCR), which can detect DNA of various Candida species in serum samples of over 50% of clinically suspected individuals without positive blood cultures, was confirmed by Alam et al. (2007) Mannan and D-BDG must be combined to prevent false positive reactions (BM *et al.*, 2013; Dheeb et al., 2023; Al-Sarraj *et al*., 2024).

## Conclusion

The present study concluded 81% positive correlation between the ELISA tests for IgM and 66% for IgG for rapid and good detection with low cost.Thus, the use of anti-mannan antibodies for the diagnosis of candidiasis should be considered and given more importance for identification purposes. So, manan antibodies can provide a more accurate and reliable determination of the condition.

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