

Association of IL-10 and TLR2 Immune Response Variations in Vaginal Candidiasis: A Molecular and Immunological Study

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ABSTRACT

Background: Vaginal candidiasis (VC) is a common fungal infection primarily caused by *Candida albicans*, affecting a significant proportion of women worldwide. The immune response plays a crucial role in controlling fungal colonization, with interleukin-10 (IL-10) and Toll-like receptor 2 (TLR2) being key regulators of inflammation and host defense mechanisms. **Objective:** This study aims to analyze the variations in IL-10 and TLR2 immune responses in patients with vaginal candidiasis, assessing their potential role in susceptibility to infection and disease severity. **Methods:** A case-control study was conducted on vaginal swab samples collected from women diagnosed with vaginal candidiasis (n=50) and healthy controls (n=50). Molecular detection of *Candida* species was performed using PCR, while serum IL-10 and TLR2 expression levels were assessed using ELISA and RT-qPCR. Statistical analysis was conducted to determine correlations between cytokine expression, fungal load, and clinical severity of infection. **Results:** The findings revealed a significant decrease in IL-10 expression among infected patients ($p < 0.05$), suggesting a weakened anti-inflammatory response in vaginal candidiasis. Conversely, TLR2 expression was upregulated, indicating an enhanced innate immune activation against fungal invasion. A strong correlation was observed between TLR2 overexpression and severe symptomatic cases, highlighting its role in host-pathogen interactions. **Conclusion:** The study underscores the differential immune response of IL-10 and TLR2 in vaginal candidiasis, with TLR2 upregulation linked to increased disease severity and IL-10 downregulation associated with impaired immune modulation. These findings may contribute to developing targeted immunotherapeutic approaches for managing recurrent vaginal candidiasis.

Keywords: Cytokines, Interleukin-10, Types of *Candida*.

INTRODUCTION

Candidiasis is a primary or secondary fungal infection caused by species of the genus *Candida* spp. This infection is either acute, moderate, or chronic. The pathogen causes a local injury in the mouth, pharynx, skin, vagina, fingers, nails and trachea. This could possibly spread inside the body, and cause lungs infections as well as gastrointestinal tract or systemic injuries [1]. Research on fungal cases and their frequency has increased in recent years; this is due to the increasing number of people with diseases that weakened the immune system over years (Diabetes Mellitus, Tuberculosis Leukemia, and Acquired Immunodeficiency Syndrome (AIDS) [2]. *C. albicans* yeast is the main cause of infection, followed by other types such as, *C. krusei*, *C. glabrata*, *C. tropicalis*, *C. norvegensis*, *C. kefyr*, *C. parapsilosis*, *C. famata* and others [3]. The immune response is the body's ability to resist harmful microbes such as fungi and bacteria that cause diseases through a complex system consisting of a group of cells, molecules and tissues called the immune system [4]. This immune system provides protection against a variety of harmful substances that invade the body and protection is one of the basic features. It can destroy foreign organisms without affecting the rest of the body's healthy cells [2]. The innate immune system uses multiple receptors to recognize fungal pathogens; the inflammatory response is controlled by interactions between these receptors. Many fungi are recognized, at least in part, by Toll-like 2 (TLR2) and IL-10 receptors. Examining the roles these receptors play together is a useful model for understanding the interaction between innate immune receptors.

MATERIALS AND METHODS

1- The current study included 150 patients of different ages at Hit

General Hospital and private women's clinics and is conducted from 15/12/2022 to 30/3/2023. Filling out a patient information form was also used in the study. 2- Blood samples: 5 ml of the patient's blood and isolated serum were taken for immunological study. Cotton swabs were used for the inner surface of the vagina of married women for microscopic and implant examination in order to confirm infection with vaginal candidiasis. 3- Immunological test: The study is also an attempt to measure the concentration of (Interleukin-10) IL-10 and TLR2 (Toll-like Receptor 2) in infected and healthy women by ELISA method [5].

Ethical Consideration

The study was conducted in accordance with the ethical principles that have their origin in the *Declaration of Helsinki*. It was carried out after getting the patients' approval. The study protocol, the subject information and consent form were reviewed and approved by the institutional ethics committee (Putting date as Ref: 55 dated 30/10/2020).

Statistical Analysis

Data were processed and examined by conducting statistical analysis SPSS version 26.0 (SPSS, IBM, Chicago, Illinois). The research outcomes were expressed as (mean, SD) t-test which was used to analyze the differences. The t-test was used to compare patient control systemic and chi-square. A value of $P < 0.05$ was statistically significant [2].

Results

Between December 2022 and March 2023, our study was

conducted on 150 patients and for different age groups. The incidence of candidiasis showed a rate of .3461% for all age groups, and the study also showed the presence of four types of yeasts. The type was the *C.*

albicans which showed 53.26%, the type *C. krusei* indicated 40.22%; while the two types *C. glabrata* and *C. tropicalis* rated 4.35% and 2.17% respectively. The age group 26-35 is the most affected one.

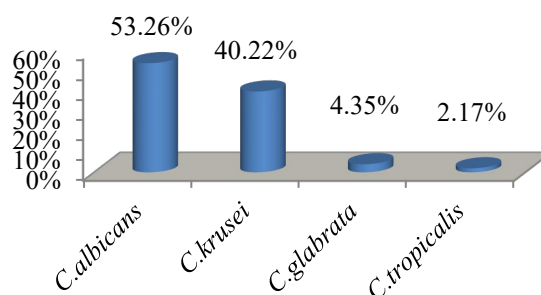


Figure 1: Percentage of Candida spp Isolates on CHROM Agar Medium

The study showed that there was a significant increase in $P < 0.05$ at the level of TLR2 and IL-10 and for all age groups. The higher levels were higher in the age group (36-45) for patients, compared to

controlling a significant difference at the level of probability. $P < 0.05$ which was measured using enzyme-bound immunoassay titration technology (ELISA).

Table 1: TLR2 and IL-10 Levels

Group	Concentration TLR2 mg\dl M±S.D	Concentration IL-10 mg\dl M±S.D
Control	5.237 ± 0.995	75.374 ± 20.920
Infection	12.828 ± 20.920	198.067 ± 131.357

*There are Significant differences at the Probability Level of $P < 0.05$

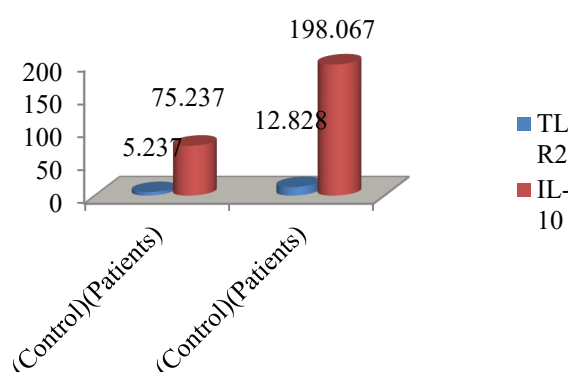


Figure 2: TLR2 and IL-10 Levels

Table 2: Evaluation of IL-10 and TLR2 Levels in Different Age Groups

Age Group	Group	Concentration TLR2 mg\dl M±S.D	Concentration IL-10 mg\dl M±S.D
15-25	Control	5.118 ± 1.044	69.867 ± 24.611
	Infection	12.192 ± 5.746	199.644 ± 136.365
26-35	Control	5.303 ± 0.673	81.969 ± 11.340
	Infection	12.593 ± 0.175	197.710 ± 144.099
36-45	Control	4.114 ± 3.788	88.157 ± 14.51
	Infection	14.125 ± 6.924	196.114 ± 128.082
46-55	Control	4.011 ± 1.957	61.391 ± 24.343
	Infection	9.137 ± 1.315	145.167 ± 15.905

*There are significant differences at the probability level of $P < 0.05$

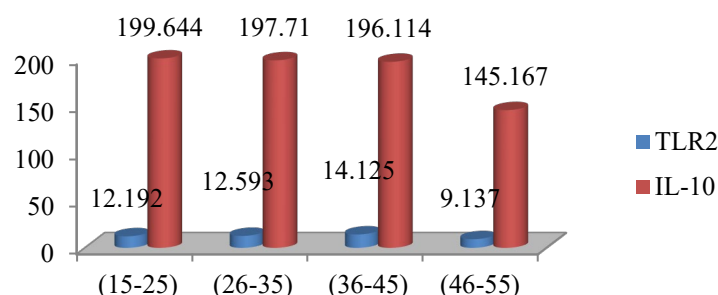
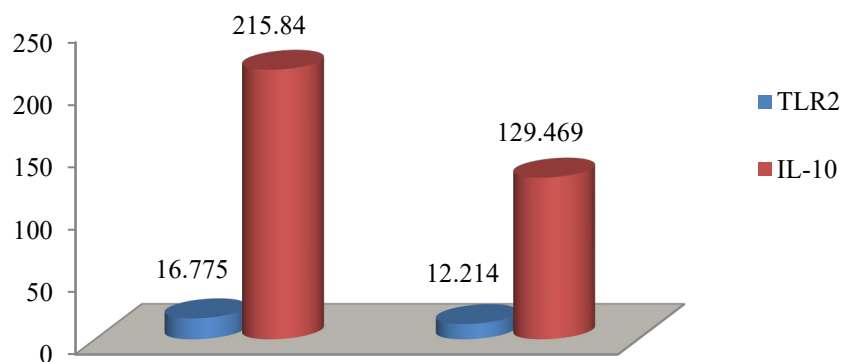


Figure 3: Evaluation of IL-10 and TLR2 Levels in Different Age Groups

Table 3: TLR2 and IL-10 Levels of Pregnant and Non-Pregnant Women

Age Group	Group	Concentration TLR2 mg\dl M±S.D	Concentration IL-10 mg\dl M±S.D
Pregnant Women	Control	5.3960 ±0.722	74.611 ± 20.861
	infection	16.775 ± 8.691	215.840 ± 141.072
Non- Pregnant Women	Control	5.244 ± 1.01	95.420 ± 2.955
	infection	12.214 ± 5.002	129.469 ± 26.589

**Figure 4:** TLR2 and IL-10 Levels of Pregnant and Non-Pregnant Women

The study also showed a significant increase of $p < 0.05$ at the level of TLR2 and IL-10 for pregnant women, compared to control (non-pregnant women) and the elevation levels of TLR2 and IL-10 of pregnant women, which were higher than in the category of non-pregnant women with vaginal candidiasis

Discussion

Candidiasis is a primary or secondary fungal infection caused by the genus *Candida spp.*, as it is the second most common infection in women and represents about 17%–42% [6]. According to our study, the prevalence rate of infection was 61.34%, which was higher than the rates reported by [7], at a rate of (49.8%). In this study, there were four species belonging to the genus *Candida*; the most prevalent of which is the genus *Candida* of the type *C. albicans*, where the number of isolates reached 49 isolates and by 54%. This type is more pathogenic between the sexes [8]. The age group (25–35) was the most susceptible to vaginal candidiasis. This result was close to what obtained [9]. It was noted that most of the women in this category are pregnant, where the percentage of pregnant women for this category reached 64.5%, and most of them were in the third trimester of pregnancy. The most important reason is that women within this age group are at the peak of their reproductive years, and their use of contraceptives, whether oral (drugs), uterine or hormonal. These matched with the findings of [10] and [11] that women during their childbearing years are more likely to develop vaginal candidiasis than other age groups. Besides, it was affirmed that pregnant women are more susceptible to candidiasis, as the incidence rate reached 66%; vagina is more sensitive and susceptible to infection. This may be due to several reasons, the most important of which is low immunity in pregnant women for a relatively long time. Furthermore, it is noted that the incidence rate increases during the last trimester of pregnancy. In order to understand the immune response to vaginal candidiasis, TLR2 and IL-10 levels were measured as they increased at fungal infection, and reduced susceptibility to candidiasis that is associated with reduced production of IL-10 [12]. The data indicate that the TLR2 mainly stimulates anti-inflammatory signaling during candidiasis as IL-10 has a strong

inhibitory effect on the defence against *Candida spp.* species. This may represent a mechanism to evade host defences, in addition to its inhibitory effects. For macrophages and neutropenia function, recent data suggest that IL-10 plays a central role in the adaptive form of immunity [13]. The increase in TLR2 in infection is due to the containment of the cell wall of *Candida albicans* chitin, dextran and aphid, in addition to molecules of intracellular RNA and RNA, which represent molecular patterns associated with pathogens that the host immune system can recognize and activate the immune response. TLR2 recognizes phosphorous mannan, while TLR7 recognizes singular RNA, and TLR2 recognizes phosphorous mannan and prevents invasion of *Candida albicans* [14]. The lack of signaling released by TLR2 increases when fungal infection occurs in the female lower reproductive tract; usually, the fungus attaches to the cells. Epithelial in patients with TLR2 deficiency, the fungus can easily cross the epithelial barrier not by sutures of *Candida albicans* that invade the deep mucosa and result in severe tissue damage [15]. This confirms that TLR2 is an important factor in controlling the progression of vaginal candidiasis, while there is an increase in IL-10 in fungal infection. Moreover, it is observed that there is an interaction between the types of *Candida* and epithelial tissue in the vagina where *Candida* during infection stimulates the immune system, which in turn stimulates the production of IL-10 [16]. Many studies indicate that inflammatory mechanisms remove the pathogen and then modify it by IL-10 before disease occurs and therefore the relative amounts required to produce cytokines at infection and inflammation are important for safe healing [17], IL-10 plays as the main immune regulator during infection with bacteria, fungi, viruses and parasites [18]. IL-10 is considered anti-vulvovaginal candidiasis; IL-10 can suppress inflammatory responses in tissues, leading to poor pathogen control and reducing fungal and acquired immune diseases by reducing T cell activity in lymph nodes and elevated levels. TLR2 in the age group (36–45), and it was shown that the older the person is, the less IL-10 is produced in the body when infected with fungi. Many factors help increase the rate of fungal infection, including insufficient awareness of the correct methods of personal hygiene and the excessive use of disinfectants, which leads to the elimination of beneficial bacteria that maintain the balance of vaginal microbes and poor

eating habits. It also contributes to an increase in the incidence rate [19], and the immune indicators TLR2 and IL-10 were also higher in the group of pregnant women with vaginal candidiasis, compared to non-pregnant infected women. As the increased levels of glycogen in vaginal secretions during pregnancy provide an excellent source of carbon, which encourages the growth of *Candida albicans*, pregnancy hormones play a role in increasing the infection of the vagina by *Candida*. As progesterone suppresses the anti-candida neutrophil activity, estrogen limits cell activity in preventing the growth of *Candida albicans* [20].

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Conclusions

When candidiasis occurs, an immune response occurs, which in turn causes an increase in the concentrations of the cytokine TLR2 and interleukin 10.

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