# Immunological, Hormonal, and Molecular Insights into Vulvovaginal Candidiasis among Non-Pregnant Women in Tikrit City, Iraq

**Duaa Ahmed Hassan Al-Kailani[1*] Sarab Waleed Hassan Al-Samarrai[1*], Saja Salim Alrahmani[1], Aseel Salah Mahmoud Akuola[1]**

[1]Department of Biotechnology, College of Applied Sciences, Samarra University

**Corresponding Author: sarab.waleed@uosamarra.edu.iq*

**Abstract**— Vulvovaginal candidiasis (VVC) is a common fungal infection with millions of women becoming victims worldwide. The aim of the study was to examine the correlation of Candida species distribution, host immune response, hormonal profile and virulence factors in non-pregnant women in Tikrit, Iraq. A cross-sectional analytical study was carried out between February to May 2025 among a total of 210 non-pregnant women between 18-45 years. Vaginal swabs and blood samples were taken. The identification of Candida was done by the use of culture and CHROMagar. ELISA was used to measure serum Estradiol, Progesterone, IL-6, IL-8, IL-17, and TNF-a. Virulence factors such as biofilm formation, phospholipase activity, proteinase activity and adhesion capacity were evaluated. The Candida species were obtained in 102 (48.6) participants. The predominant one was C. albicans (66.7%), then C. glabrata (21.6%). VVC women had much higher serum levels of Estradiol (192 +- 34 vs. 121 +- 27 pg/mL, $p < 0.001$) and IL-6, IL-8 and IL-17 levels than controls. Fifty-eight percent of isolates were found to be strong biofilm formers. The vaginal pH was highly raised in infected females (4.9 +- 0.4 vs. 4.2 +- 0.3). Candida adhesion ability had a positive relationship with Estradiol levels ($r = 0.62$, $p < 0.01$). VVC in Tikrit is linked with high estrogen, disturbed vaginal microenvironment, high levels of inflammatory cytokines and high Candida virulence. Patient management can be enhanced by including immunological, hormonal markers into the diagnostic strategies.

**Keywords:** Biofilm, Candida albicans, Cytokines, Estrogen, Iraq, Tikrit, Virulence factors, Vulvovaginal candidiasis.

## 1. INTRODUCTION

Vulvovaginal candidiasis (VVC) is one of the most common fungal infections in women all over the world, where epidemiologic researches reveal that about 75 percent of women have at least one episode of infection, and 40-50 percent experience recurrent episodes over the course of their reproductive lives [1,2]. It is a condition associated with the presence of vulvar pruritus and burning, dyspareunia, and abnormal vaginal discharge which has a significant effect on the quality of life and the psychological well-being of women [3].

More than 200 species make the genus Candida, and Candida albicans is the most common etiological agent among all Candida species and contributes to 80-90% of VVC cases worldwide [4]. Nevertheless, the advent of non-albicans Candida (NAC) species, such as C. glabrata, C. tropicalis, and C. krusei, have become the topic of much interest as they become more common and resistant to traditional antifungal drugs in a kind of inherent or acquired resistance [5]. This epidemiological transition requires proper identification of the species to treat them properly.

The pathogenesis of VVC is characterized by a multifactorial interaction of virulence factors of the pathogen and the defense of the host. Candida organisms have several virulence factors such as the adhesion to epithelial cells, morphological switching between the yeast and the hyphae, hydrolytic enzyme secretion (phospholipases, proteinases and lipases), and biofilm formation [6,7]. These virulence factors help the organism to colonize, invade and survive in the vaginal mucosa by avoiding the host immunity.

Estrogen is an important hormone in regulating vaginal pathology and Candida infection. The hormone affects the vaginal epithelial thickness, glycogen deposition and expression of epithelial receptors to mediate Candida adhesion [8]. High concave rates of estrogen boosts the colonization of fungi by heightening epithelial receptivity and disabling local cell-mediated immunity. Moreover, estrogen also encourages Candida hypha growth, and enhances the expression of adhesins and invasins [9].

The immune responses of the host to Candida infection include both the innate and the adaptive response. Belonging to the pattern recognition receptors (PRRs), vaginal epithelial cells identify Candida, which provokes the secretion of pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-17 (IL-17), and tumor necrosis factor-alpha (TNF-a) [10]. Th17 cells produce IL-17, which is a vital part of anti-Candida immunity because it attracts neutrophils and induces the synthesis of antimicrobial peptides [11]. Malfunction of this cytokine network plays a role in symptomatic infection and recidivism.

The natural resistance to Candida overgrowth is facilitated by the vaginal microenvironment that features acidic pH (3.8-4.5) that is upheld by Lactobacillus species [12]. Change in vaginal pH, which is usually caused by the changes in hormones, the use of antibiotics, or sex, provides an environment that will promote the growth of Candida and its symptomatic infection.

Although the global burden of VVC and its high effects on the female organism are quite overwhelming, very limited comprehensive studies on the disease (hormonal, immunological, and molecular) are present in the Middle East, specifically in Iraq [13]. The local epidemiology and pathogenic mechanisms should be understood to formulate specific prevention and treatment measures. As such, the paper had the objective of examining the prevalence and species distribution of Candida among suspected non-pregnant women with VVC in Tikrit, Iraq and to examine the relationship between hormonal levels, immune markers, and fungal virulence factors.

## 2. METHODS

### 2.1. Study Design and Setting

The study was a cross-sectional analysis study and was performed between February to May 2025 in Salah Al-Din Teaching hospital and some chosen private gynecology clinics in Tikrit city, Salt Lake Governorate in Iraq. The Research Ethics Committee of Tikrit University College of

Medicine endorsed the study protocol and all participants were provided with the informed consent in written form.

### 2.2. Study Population

There were 210 non-pregnant women aged between 18-45 years who were enrolled in the study. The sample size was divided into two groups, symptomatic women who had clinical manifestations indicative of VVC (vulvar pruritus, burning, abnormal discharge, dyspareunia) and those whose symptoms were entirely absent (control group). The exclusion criteria were as follows: pregnancy, lactation, menstruation during the sampling period, the use of antibiotics or antifungals within the last two weeks, immunosuppressive therapy, diabetes mellitus, and HIV infection.

### 2.3. Sample Collection

Each participant was put under aseptic conditions, and using sterile cotton swabs, two vaginal swabs were taken. Direct microscopy and culture were conducted using the first swab and the second kept in sterile saline awaiting the analysis. Also, 5 mL of venous blood was taken to plain tubes to separate serum. The samples of serum were kept at -20degC in anticipation of analysis. The calibrated pH indicator strips (range 3.6-6.1) were placed on the lateral vaginal wall to measure vaginal pH.

### 2.4. Culture and Identification

Microscopic examination of vaginal swabs was done using wet mount preparation of 10 percent potassium hydroxide (KOH). A 24-48 hours incubation on Sabouraud Dextrose Agar (SDA), supplemented with chloramphenicol (50 mg/L) at 37degC was used to inoculate swabs. Colonies with a typical yeast morphology were resubcultured on CHROMagar Candida (CHROMagar Company, France) to identify the species using colony color. C. albicans gave green colonies, C. glabrata gave pink to purple colonies, C. tropicalis gave blue colonies and C. krusei gave pink fuzzy colonies.

### 2.5. Hormonal and Immunological Assays

Estradiol (E2) and Progesterone serum levels were determined with the help of commercial ELISA kits (Monobind Inc., USA). The levels of IL-6, IL-8, IL-17 and TNF-a in serum were determined with the help of sandwich ELISA kits (R&D Systems, USA). Each sample underwent a twofold determination of which the mean reading of a pair of determinations was analyzed. A microplate reader was used to measure absorbance at 450 nm and concentrations were determined by use of standard curves.
An evaluation of virulence factors was conducted to assess the virulence of the identified bacterium and its ability to induce disease in humans.

### 2.6. Viral Factor Assessment

Virulence factor assessment was done to determine the virulence of the identified bacteria and its capability in causing disease in humans.

Crystal violet assay was used to measure the biofilm formation in 96-well polystyrene microtiter plates. Isolates were classified as non-producers (OD [?] 0.1), weak producers (OD 0.1< OD [?]

0.2), moderate producers (OD 0.2< OD [?] 0.4), or strong producers (OD [?] 0.4). The activity of phospholipase was established in agar medium using egg yolk and expressed in Pz value (colony diameter/colony diameter plus the precipitation zone). The activity of proteinase was determined using the bovine serum albumin (BSA) agar. The adhesion capability was measured with the help of buccal cells of healthy individuals after measurements of the number of adherent yeast cells per 100 cells of the epithelial cell mass were used to calculate the adhesion index.

### 2.7. Statistical Analysis

The SPSS version 26.0 (IBM Corp., USA) was used to analyze the data. The ShapiroWilk test was used to test the normal distribution of data. The continuous variables were displayed in mean +- standard deviation (SD) and compared with student t-test. Frequencies and percentages were used to show categorical variables and Chi-square test was used to analyze them. The continuity variables were evaluated through Pearson correlation coefficient to determine the relationship between the variables. Binary logistic regression was conducted to determine predictors of VVC which are independent. The p-value that was taken to be significant was 0.05.

## 3. RESULTS

### 3.1. The Demographic and Clinical Characteristics Comprise

This study employed 210 non-pregnant women. The average age was 29.4 +- 7.2 years (18-45 years). Most of the participants were married (78.6%), and 45.2% of them had a history of the past VVC episodes. The most widespread presenting complaints included in symptomatic females were vulvar pruritus (89.2%), abnormal vaginal discharge (76.5%), burning sensation (62.7%), and dyspareunia (41.2%). The descriptions of demographics are given in Table 1.

Table 1. Demographic and Clinical Characteristics of the participants in the study.

| Characteristic | Infected (n=102) | Control (n=108) | p-value |
|---|---|---|---|
| Age (years), mean ± SD | 29.8 ± 6.9 | 29.0 ± 7.5 | 0.42 |
| Married, n (%) | 82 (80.4%) | 83 (76.9%) | 0.53 |
| Previous VVC history, n (%) | 58 (56.9%) | 37 (34.3%) | <0.01* |
| Vaginal pH, mean ± SD | 4.9 ± 0.4 | 4.2 ± 0.3 | <0.001* |
| pH > 4.5, n (%) | 75 (73.5%) | 20 (18.5%) | <0.001* |

*Statistically significant ($p < 0.05$)

### 3.2. Prevalence and Species Distribution

One hundred and twenty (102) participants out of a total of 210 yielded Candadae species. The C. albicans species was the most dominant species with 68 (66.7) isolates and C. glabrata species with 22 (21.6) isolates and 8 (7.8) and C. lusitaniae species with 4 (3.9) isolates, respectively. It is worth noting that C. krusei was not the only one in our population of study. Table 2 shows the distribution of the species.

Table 2. The Distribution of the Candida Species among the Positive Cases (n=102)

| Candida Species | Number | Percentage (%) |
|---|---|---|
| *C. albicans* | 68 | 66.7 |
| *C. glabrata* | 22 | 21.6 |
| *C. tropicalis* | 8 | 7.8 |
| *C. lusitaniae* | 4 | 3.9 |
| **Total** | **102** | **100.0** |

### 3.3. Hormonal Levels

Hormonal analysis of serum showed that women having VVC had much higher Estradiol concentration (192 +- 34 pg/mL) than controls (121 +- 27 pg/mL, $p < 0.001$). On the same note, the level of Progesterone was also high in infected women (2.1 +- 0.6 ng/mL) relative to the controls (1.3 +- 0.4 ng/mL, $p < 0.01$). Comparison of hormonal profile is provided in Table 3.

Table 3. Comparison of the Serum Hormonal Levels between the Groups.

| Parameter | Infected (n=102) | Control (n=108) | p-value |
|---|---|---|---|
| Estradiol (pg/mL) | 192 ± 34 | 121 ± 27 | <0.001* |
| Progesterone (ng/mL) | 2.1 ± 0.6 | 1.3 ± 0.4 | <0.01* |

*Statistically significant ($p < 0.05$)

### 3.4. Immunological Markers

The cytokine analysis of serum showed a high level of all the inflammatory markers in women with VVC when compared to control. The level of IL-6 were also significantly greater in infected women (18.4 +- 4.1 pg/mL) than controls (7.2 +- 2.3 pg/mL, $p < 0.001$). There was the strongest increase in IL-8 of the measured cytokines (45.2 +- 8.7 vs. 19.5 +- 5.1 pg/mL, $p < 0.001$). The IL-17 and TNF-a were also found to be immensely elevated in the women who were infected. Table 4 shows detailed cytokine profiles.

Table 4. Serum Cytokine Level Comparison across Groups.

| Cytokine (pg/mL) | Infected (n=102) | Control (n=108) | p-value |
|---|---|---|---|
| IL-6 | 18.4 ± 4.1 | 7.2 ± 2.3 | <0.001* |
| IL-8 | 45.2 ± 8.7 | 19.5 ± 5.1 | <0.001* |
| IL-17 | 22.6 ± 5.3 | 9.1 ± 2.8 | <0.001* |
| TNF-α | 14.3 ± 3.2 | 6.8 ± 1.9 | <0.001* |

*Statistically significant ($p < 0.05$)

### 3.5. Virulence Factors

Virulence factors evaluation of the Candida isolates indicated a high pathogenic potential. The biofilm-forming capacity was found to be high in 59 (57.8) of the isolates with C. albicans having the highest biofilm-forming capacity of 64.7% strong biofilm producers versus NAC species at 44.1% strong biofilm producers. The 64 (62.7) and 71 (69.6) isolates were found to have high phospholipase and proteinase activity respectively. Table 5 shows the distribution of the virulence factors.

Table 5. Virulence Factors Distribution in Candida Isolates.

| Virulence Factor | *C. albicans (n=68)* | NAC (n=34) | Total (n=102) |
|---|---|---|---|
| Strong Biofilm | 44 (64.7%) | 15 (44.1%) | 59 (57.8%) |
| High Phospholipase | 48 (70.6%) | 16 (47.1%) | 64 (62.7%) |
| High Proteinase | 52 (76.5%) | 19 (55.9%) | 71 (69.6%) |
| High Adhesion Index | 46 (67.6%) | 14 (41.2%) | 60 (58.8%) |

*NAC: Non-albicans Candida*

### 3.6. Correlation and Regression Analysis

The Pearson correlation analysis between the serum Estradiol levels and Candida adhesion index ($r = 0.62$, $p < 0.01$), biofilm formation ($r = 0.48$, $p < 0.01$) and vaginal pH ($r = 0.41$, $p < 0.05$) were found significant and positive after the Pearson correlation analysis was carried among infected women (n=102). There was a positive relationship between the levels of IL-17 with biofilm formation ($r = 0.52$, $p < 0.01$) and phospholipase activity ($r = 0.39$, $p < 0.05$). Binary logistic regression analysis revealed that elevated levels of Estradiol (OR = 2.84, 95% CI: 1.72-4.69, $p < 0.001$), elevated levels of IL-17 (OR = 2.31, 95% CI: 1.45-3.68, $p < 0.01$) and vaginal pH above 4.5 (OR = 3.12, 95% CI: 1.89-5

## 4. DISCUSSION

The work gives detailed information about the epidemiology, immunological reactions, hormonal relationship, and virulence factors of Candida species that cause vaginal candidiasis of the vulva in non-pregnant women in Tikrit, Iraq. The results add to the insufficient literature on VVC in the Middle East area and have significant clinical management implications. The general Candida colonization rate of our study population comprised 48.6% which is consistent with the world estimates of between 20 and 50 per cent in women of reproductive age [1,2]. The prevalence is significantly large as compared to that recorded in some developed countries but similar to other Middle Eastern and developing nations. The high levels can be explained by climatic conditions, hygiene, care-seeking habits and socioeconomic factors in the area. C. albicans was the most common species (66.7%), which is confirmed as the major etiological agent of VVC across the world [4]. Nonetheless, the high percentage of NAC species (33.3%), and C. glabrata in particular (21.6 percent) is interesting, and complies with the global trend of rising NAC infections [5,14]. The clinical implications of this finding are profound because the C. glabrata has a decreased susceptibility to the administration of azole antifungals and might have to be treated with other

interventions. Although C. lusitaniae (3.9%), though rare, is a topic to be considered, it is inherently resistant to amphotericin B [15].

Our results reveal that there is a high relationship between high levels of serum Estradiol and VVC. The concentration of hormones in infected women was much higher than that of controls. This observation supports the known fact that estrogen enhances Candida colonization and infection [8,9]. Estrogen increases the vaginal epithelial glycogen, which has a rich environment that supports the growth of fungi. In addition, estrogen increases the expression of epithelial cell surface receptors to enable Candida adhesion that was found to be significantly positive ($r = 0.62$) between Estradiol levels and adhesion index in our study. The disrupted normal vaginal ecosystem is manifested by the high vaginal pH (4.9 +- 0.4) of the infected women, as opposed to the controls (4.2 +- 0.3) [12]. Lactobacillus-produced lactic acid creates an acidic vaginal environment which is a natural protection mechanism against Candida overgrowth. The given finding of pH being the independent predictor of VVC (OR = 3.12) can highlight the role of vaginal homeostasis in preventing infection. The immunologic examination showed that women with VVC have high levels of pro-inflammatory cytokines (IL-6, IL-8, IL-17, TNF-a) [10,11]. The observations indicate stimulation of host natural and adaptive immune response to Candida infection. Th17 cell-produced IL-17 is essential in mucosal anti-Candida immunity. The high level of Th17 infection in our infected group and the fact that it is an independent predictor of VVC (OR = 2.31) demonstrate the significance of Th17 pathway in VVC pathogenesis.

The significant increase in the IL-8, which is a strong neutrophil chemoattractant, indicates strong neutrophil-mediated inflammatory response to Candida infection. Although neutrophils play a crucial role in clearing fungi, an excess of neutrophil infiltration is the cause of the clinical manifestations of VVC, such as inflammation of the vulva, erythema and tissue destruction [16]. This contradictory nature of immune responses in protection and pathology is the key to the symptomatology of VVC. The detection of virulence factors showed that Candida isolates had high pathogenic potential [6,7]. The formation of biofilms is also notable because it grants their resistance to antifungal agents and host immune responses which add to their failure to be treated and their repeated occurrence [17]. The increased pathogenic potential of C. albicans is confirmed by the greater number of virulence factors it has over its NAC counterparts. The correlation occurring between the Estradiol levels and the expression of virulence factors (biofilm formation, adhesion) is positive and indicates that the hormonal milieu can not only promote Candida colonization but also increases the fungal virulence [9].A number of limitations are to be noted in this study. The cross-sectional design does not allow defining any causal relationship between the examined variables. The sample was selected in one geographic area thereby restricting the generalizability. Also, antifungal susceptibility was not carried out which would have been useful in treatment directions. Prospective follow-up should be incorporated in future studies in order to have more knowledge of VVC recurrence patterns.

## 5. CONCLUSIONS

This paper shows that vulvovaginal candidiasis in Tikrit, Iraq is a complex disease with a high prevalence of C. albicans and high levels of NAC species, in which C. glabrata is a dominant species. High levels of estrogen, a change of vaginal pH, high levels of pro-inflammatory cytokines, and a high degree of expression of Candida virulence factors are also strongly related to the infection. The combination of hormonal and immunological parameters into the diagnostic

algorithms could enhance diagnosing the high-risk women in VVC and directing the individual approach to the treatment. The high percentage of NAC species indicates the necessity to identify the species of the antifungal correctly to make the right choice.

**Acknowledgments**
The authors would also like to appreciate the staff members of Salah Al-Din Teaching Hospital and the participating private gynecology clinics to help them in the collection of samples. It is also our gratitude to all the women who took part in this study.

**Funding**
No specific grant was given to this research by any of the funding agencies in the public, commercial, or not-for-profit sector.

**Conflict Of Interest**
The authors indicate that the study had been carried out without any commercial or financial interests that may be interpreted as possible conflict of interest.

## 6. REFERENCES

[1] Sobel JD. Vulvovaginal candidosis. Lancet. 2007;369(9577):1961-1971.

[2] Gonçalves B, Ferreira C, Alves CT, Henriques M, Azeredo J, Silva S. Vulvovaginal candidiasis: Epidemiology, microbiology and risk factors. Crit Rev Microbiol. 2016;42(6):905-927.

[3] Yano J, Sobel JD, Nyirjesy P, et al. Current patient perspectives of vulvovaginal candidiasis: incidence, symptoms, management and post-treatment outcomes. BMC Womens Health. 2019;19(1):48.

[4] Willems HME, Ahmed SS, O'Neill J, Rber MR, Jabra-Rizk MA. Vulvovaginal candidiasis: a current understanding and burning questions. J Fungi. 2020;6(1):27.

[5] Makanjuola O, Bongomin F, Fayemiwo SA. An update on the roles of non-albicans Candida species in vulvovaginitis. J Fungi. 2018;4(4):121.

[6] Naglik JR, Challacombe SJ, Hube B. Candida albicans secreted aspartyl proteinases in virulence and pathogenesis. Microbiol Mol Biol Rev. 2003;67(3):400-428.

[7] Harriott MM, Lilly EA, Rodriguez TE, Fidel PL Jr, Noverr MC. Candida albicans forms biofilms on the vaginal mucosa. Microbiology. 2010;156(Pt 12):3635-3644.

[8] Fidel PL Jr. History and update on host defense against vaginal candidiasis. Am J Reprod Immunol. 2007;57(1):2-12.

[9] Cheng G, Yeater KM, Hoyer LL. Cellular and molecular biology of Candida albicans estrogen response. Eukaryot Cell. 2006;5(1):180-191.

[10] Rosati D, Bruno M, Jaeger M, Ten Oever J, Netea MG. Recurrent vulvovaginal candidiasis: An immunological perspective. Microorganisms. 2020;8(2):144.

[11] Conti HR, Shen F, Nayyar N, et al. Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. J Exp Med. 2009;206(2):299-311.

[12] Bradford LL, Ravel J. The vaginal mycobiome: A contemporary perspective on fungi in women's health and diseases. Virulence. 2017;8(3):342-351.

[13] Denning DW, Kneale M, Sobel JD, Rautemaa-Richardson R. Global burden of recurrent vulvovaginal candidiasis: a systematic review. Lancet Infect Dis. 2018;18(11):e339-e347.

[14] Sobel JD, Sobel R. Current treatment options for vulvovaginal candidiasis caused by azole-resistant Candida species. Expert Opin Pharmacother. 2018;19(9):971-977.

[15] Pappas PG, Kauffman CA, Andes DR, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;62(4):e1-e50.

[16] Peters BM, Yano J, Noverr MC, Fidel PL Jr. Candida vaginitis: when opportunism knocks, the host responds. PLoS Pathog. 2014;10(4):e1003965.

[17] Nobile CJ, Johnson AD. Candida albicans biofilms and human disease. Annu Rev Microbiol. 2015;69:71-92.

[18] Mayer FL, Wilson D, Hube B. Candida albicans pathogenicity mechanisms. Virulence. 2013;4(2):119-128.

[19] Cassone A. Vulvovaginal Candida albicans infections: pathogenesis, immunity and vaccine prospects. BJOG. 2015;122(6):785-794.

[20] Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007;20(1):133-163.

[21] Achkar JM, Fries BC. Candida infections of the genitourinary tract. Clin Microbiol Rev. 2010;23(2):253-273.

[22] Foxman B, Muraglia R, Dietz JP, Sobel JD, Wagner J. Prevalence of recurrent vulvovaginal candidiasis in 5 European countries and the United States: results from an internet panel survey. J Low Genit Tract Dis. 2013;17(3):340-345.

[23] Gaffen SL, Hernández-Santos N, Peterson AC. IL-17 signaling in host defense against Candida albicans. Immunol Res. 2011;50(2-3):181-187.

[24] Moyes DL, Wilson D, Richardson JP, et al. Candidalysin is a fungal peptide toxin critical for mucosal infection. Nature. 2016;532(7597):64-68.

[25] Wächtler B, Citiulo F, Jablonowski N, et al. Candida albicans-epithelial interactions: dissecting the roles of active penetration, induced endocytosis and host factors on the infection process. PLoS One. 2012;7(5):e36952.