

RESEARCH PAPER

The Burden and Incidence of Vulvovaginal Candidiasis and its social impacts, in Woman's of Erbil City, Kurdistan Region of Iraq

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ABSTRACT:

Candida albicans is a yeast, type of fungus. It is typically found in little quantities in intestines, skin, genital tract and mouth as a normal flora. Often when *Candida* is off-balance, the yeast overgrows and causes infection. Many women of childbearing age suffer from vulvovaginal candidiasis (VVC). This study was conducted at Women clinics and Maternity teaching hospital in Erbil city, Iraq. A total of Sixty-five specimens from female patients collected to assess the incidence of yeast infection in women diagnosed with vaginitis by an Obstetrician and gynecologist between 10th November 2021 and 15th March 2022. Experimental and laboratory work for the present study was conducted in Microbiology laboratories of the Research Center of Salahaddin University-Erbil (SUE). The results showed were strong biofilm formers (32.1%), followed by moderated and weak (10.7%) for each type. The (MIC) for the mixture of zinc oxide nanoparticles (ZnO-NPs) with fluconazole and nystatin were 0.059 and 0.065 µg/mL, respectively and (MIC) for fluconazole and nystatin were 0.077 and 0.071 µg/mL. These findings indicated that the mixture of ZnO-NPs with fluconazole or nystatin had a significantly greater anti-fungal impact on *C. albicans* than fluconazole or nystatin alone (P=0.001). This study is the first that shaded light on the burden of *Candida* infection on the life and progress of families in Erbil City, Kurdistan Region of Iraq. Objective is determining the effectiveness of antifungals that are used for this condition, as well as the expenses spent on treating the condition and the impact of the disease on society

KEYWORDS: *Candida albicans*, Vaginal infection, ZnO-NPs, Fluconazole, Nystatin.

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1.INTRODUCTION :

The female vaginal ecology is hypothesized to have been influenced by co-evolutionary processes between the host of human and the particular microbiome over time. A wide variety of microorganisms can be found in vaginal secretions, and the host supplies them with nutrients for growth and development. Changes in the vaginal environment due to disruptions in the vaginal interaction with the microbiomes, increase the chance of acquiring diseases. This includes preterm birth, fungal infections, bacterial vaginosis, sexually transmitted infections, etc. (Gupta *et al.*, 2019).

Candida species are responsible for the majority of common vaginal diseases, which include conditions from mild to severe infections (Chatzivasilieiou and Vyzantiadis, 2019).

Candida species, most commonly *Candida albicans*, are responsible for the vaginal fungal infection known as vulvovaginal candidiasis (VVC)

(Gudia, Eswi and Hassan 2019). The transition of *C. albicans* from commensalistic to pathogenic form is still to be understood, despite the fact that candidiasis is a frequent illness affecting women (Roselletti *et al.* 2019). Approximately seventy-five percent of all women will experience VVC at some point in their lives, and between 40% and 50% will have a recurrence (Czechowicz *et al.*, 2022).

Furthermore, some women loss their self-esteem and confidence, difficulties with their intimate relationships and sexual life and impairment. This could cause difficulties in engaging in regular physical activities. Other systemic symptoms, such as depression and anxiety, have a significant

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effect on the life quality of affected women (Van Riel *et al.* 2021). Women suffering from recurrent vulvovaginal candidiasis (RVVC) make them feel 'dirty' and that partner relations become strained, as suspicions arise regarding the faithfulness of each partner (Sobel and Nyirjesy, 2021).

Different medications have been reported for the treatment of vulvovaginal candidiasis, including Oral Ibrexafungerp (Azie *et al.*, 2020), Oteseconazole (Sobel and Nyirjesy, 2021), Ibrexafungerp versus placebo (Schwebke *et al.*, 2022) and Cinnamaldehyde (Chen *et al.*, 2019). Moreover, as an alternative method for dealing with VVC, p-coumaric acid put into liquid crystalline systems has shown promised results (Ferreira *et al.*, 2021). The VVC model demonstrated a reduction in fungal infection when treated with chitosan-gel containing *Mitracarpus frigidus* methanolic extract, suggesting that this may be an effective antifungal formulation for VVC treatment (Campos *et al.*, 2020). It has been reported that the essential oil of clove, encapsulated in a polysaccharide-based nanocarrier, is a novel approach for therapy of VVC (da Silva Campelo *et al.*, 2021). Alum is the natural products that used as an anti-Candida agent (Babadi *et al.*, 2019). Nanotechnology is a recent and contemporary branch of science that focuses on the production of new nanoparticles and nanomaterials with dimensions ranging from 1 to 100 nanometers. The combination of ZnO-NPs with antifungals used in this study was significantly more effective than when they have been used alone, with a significant correlation between time of resulting and effectiveness This study found that Recurrent Vulvovaginal Candidiasis (RVVC) has a negative effect on life of many women, with high rates of daily visit clinics due to education level, person hygiene, and psychological state. The total cost per day in all clinics is 56,837,550 IQD, and per year is 11,822,210,400 IQD. These costs are a burden on families and the financial budget for each house The objective of the current study is to assess the prevalence of *Candida* vaginal infection, its impact on the social life of females, as well as to address a new strategy for better and faster treatment for such chronic illness.

2.Materials and methods,

2.1Patients

This is a prospective survey conducted at Women clinics and Maternity teaching hospital in Erbil city, Iraq, to assess the incidence of yeast infection in women diagnosed with vaginitis by an Obstetrician and gynecologist between 10th November 2021 and 15th March 2022. Experimental and laboratory work for the present study was conducted in the Microbiology laboratories of the Research Center of Salahaddin University-Erbil (SUE).

2.2Inclusion criteria

Each woman who participated in this study was provided with a written informed consent form. In addition to every woman who had experienced a vaginal infection

2.3Exclusion criteria

Women who did not complete the entire information sheet for this investigation. Women who were prescribed antibiotics within the previous week were omitted. Any women who had inconstant with inclusion criteria.

2.4Methodology

2.4.1Sample collection

This study involved 65 women's vaginal swabs taken with sterile cotton swabs and 24-hour media exchanges. For the diagnosis, swab samples were collected, packaged, and sent to the microbiology laboratories at the research center of Salahaddin University-Erbil (SUE). In the laboratories, the samples were cultured on various cultivation and differential media for 24 hours at 37°C.

2.4.2Isolation and Identification of Samples

In this survey, all specimens were culture on selective media, including Sabouraud Dextrose Agar (SDA), CHROM agar™ *Candida*, Roswell Park Memorial Institute (RPMI) and Yeast Extract Peptone-Dextrose (YPD).

2.4.3Biofilm formation test (Roche XTT procedure) :

The XTT reagent (200 µl) was activated using activator (0.1ml for every 5ml of XTT reagent). The reagent was added to each biofilm well in the plate and 3 blank wells (no biofilms control). The plate then covered with aluminum foil and incubated at 37° for 2 hours .

(adapted from Chandra *et al.*, 2001 and Pierce *et al.*, 2008)

2.4.4Antifungal Testing

The E-test was comprised an immobilized gradient of concentrations of antibiotics and was

utilized to assess the Minimum Inhibitory Concentration (MIC) of antibiotics and antifungal drugs.

2.4.5 Nanoparticles:

The micro broth dilution method was used to assess the MIC of ZnO-NPs against standard strains of *Candida albicans* in 96 sterile plates. The semi-McFarland turbidity was 1.5×10^8 CFU/ml, we used zinc particles with antifungals. Zinc was diluted with DMSO (Di Methyl Sulf Oxide).

2.5 Statistical Analysis

Using Microsoft Excel 2021 and SPSS (IBM version 26.0), the data were analysed. In this study, results were provided as mean $\pm$ SD and frequencies as percentages. The Chi-square test, the student dependent T-test, and the independent T-test were utilized to compare distributed groups. Probability levels less than 0.05 were deemed physiologically significant, and p-values less than 0.01 were deemed extremely significant.

3. Results and Discussion

3.1 Results

In this study, sixty-five (65) specimens (vaginal swabs) were gathered between November 2021 and May 2022 from women with vaginitis in Erbil, Iraq. Specimens were cultured on SDA, which is a selective medium used to differentiate and detect the fungi such as dermatophytes, yeasts, and a numerous of other both pathogenic and nonpathogenic fungi. On SDA, molds grow as varied color-filamentous colonies, whereas yeasts appeared as white to creamy colonies. In particular, *Candida albicans* appears as cream-colored pasty colonies after incubation. The colonies give out a yeasty odor, and the budding cells are visible under a microscope (both stained and unstained).

On CHROM agar™ (figure 1) *Candida*, *C. glabrata* colonies were smooth, medium in size, and pink in color with a darker mauve center; *C. tropicalis* colonies were smooth, medium in size, and blue to dark metallic in color; *C. krusei* percentage.

colonies were flat, large in spread, rough to created, and pink in color; *C. albicans* colonies were smooth, medium in size, and green to dark metallic green in color and other species colonies were white to pink in color. Biofilm formation presented in the chart 1

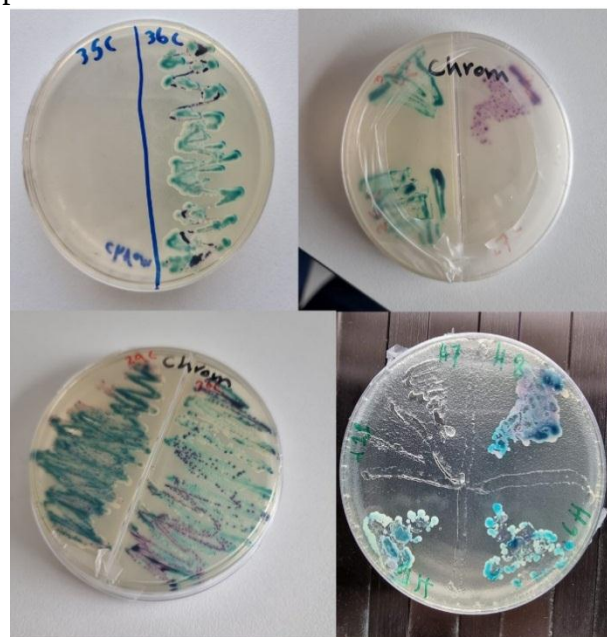


Figure1: samples on Chrom Agar

As presented in the table 1, most of the isolated *Candida* spp. in this study from vaginal swabs formed strong biofilm (32.1%), followed by moderated and weak biofilm (10.7%) for each type.

Table 1 The biofilm formation assay among patients

Variable		Frequenc y	Percen t
Biofilm Formation	Weak	6	10.70 %
	Moderat e	6	10.70 %
	Strong	18	32.10 %
	Total	30	53.60 %
No Biofilm Formation		26	46.40 %
Total		65	100%

Data presented as Count (N) is number of patients and (%) is

Biofilm Formation

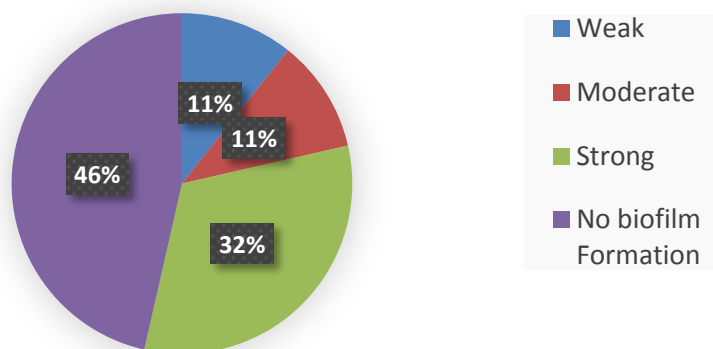


Chart 1: The biofilm formation assay among patients

As presented in the table 2, the combination of ZnO-NPs with each antifungal were better than it alone, with a significant correlation between time of resulting ($P < 0.001$).

Table 2 The statics of Antifungal groups in this study

Antibiotics	Groups	N	Mean	SD	SEM	Sig.
FLZ and ZnOnp	Post	24	0.516	0.628	0.128	0.000 ^{H.S.}
	Pre	24	0.702	0.861	0.176	
FLZ	Post	24	0.238	0.162	0.033	0.365 ^{N.S.}
	Pre	24	0.193	0.103	0.021	
NYS and ZnOnp	Post	24	0.394	0.472	0.096	0.000 ^{H.S.}
	Pre	24	0.613	0.763	0.156	
NYS	Post	24	0.314	0.446	0.091	0.000 ^{H.S.}
	Pre	24	0.297	0.301	0.062	

* Statistically, the $P < 0.001$ difference was significant when compared to antifungal-alone groups.

In the table 3, the MIC ($\mu\text{g/mL}$) of the antifungal utilized in this study for the selected specimens is reported. The minimum inhibitory concentrations (MIC) for the mixture of ZnO-NPs with fluconazole and nystatin were 0.059 and 0.065 $\mu\text{g/mL}$, respectively. These results indicated that the mixture of ZnO-NPs with fluconazole or nystatin had a significantly greater antifungal impact on *C. albicans* than fluconazole or nystatin alone ($P = 0.001$).

The minimum inhibitory concentrations (MIC) of the results of the Enzyme-linked immunosorbent assay (ELISA) device was determined and it was mentioned in the form of a chart for each subject, as follows; in chart 2(MIC) of Floconazole, and floconazole with zinc oxide in chart 3, in chart 4 nystatin & zincoxide, and nystatin alone in last chart 5

Table 3 Determination of MIC of antifungal used in this study

Sample	FLZ and ZnOnp	FLZ	NYS and ZnOnp	NYS
Sample 4	Field 6 MIC +++ (0.094)	Field 3 MIC +++ (0.077)	Field 6 MIC +++ (0.086)	Field 6 MIC +++ (0.085)
Sample 30	Field 6 MIC +++ (0.059)	Field 6 MIC +++ (0.079)	Field 6 MIC +++ (0.079)	Field 7 MIC +++ (0.077)
Sample 37	Field 5 MIC ++ (0.080)	Field 5 MIC +++ (0.120)	Field 6 MIC +++ (0.065)	Field 7 MIC +++ (0.071)

* Statistically, the $P < 0.001$ difference was significant when compared to antifungal-alone groups.

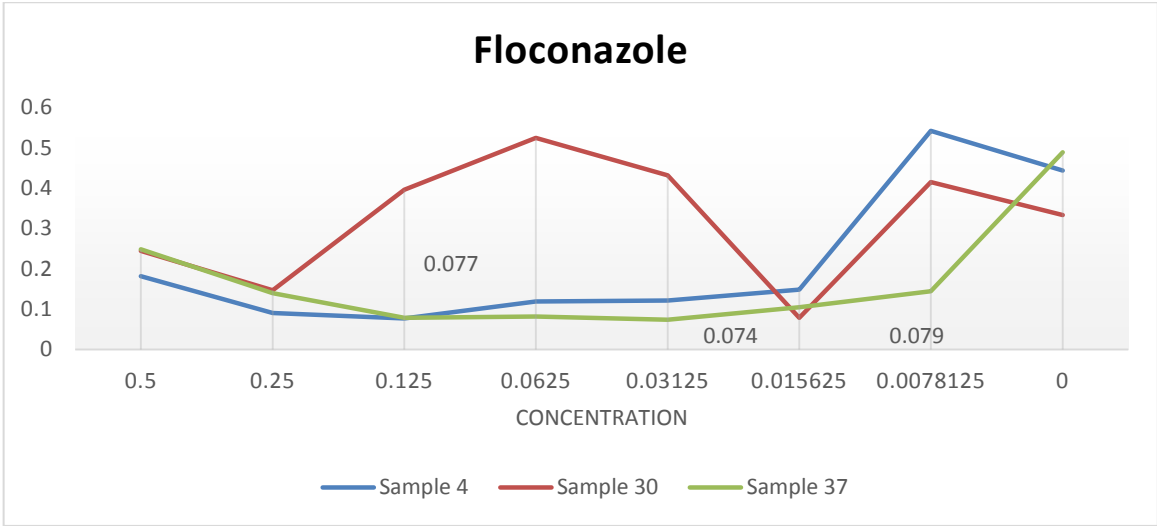


Chart 2: Determination of MIC of Floconazole in this study

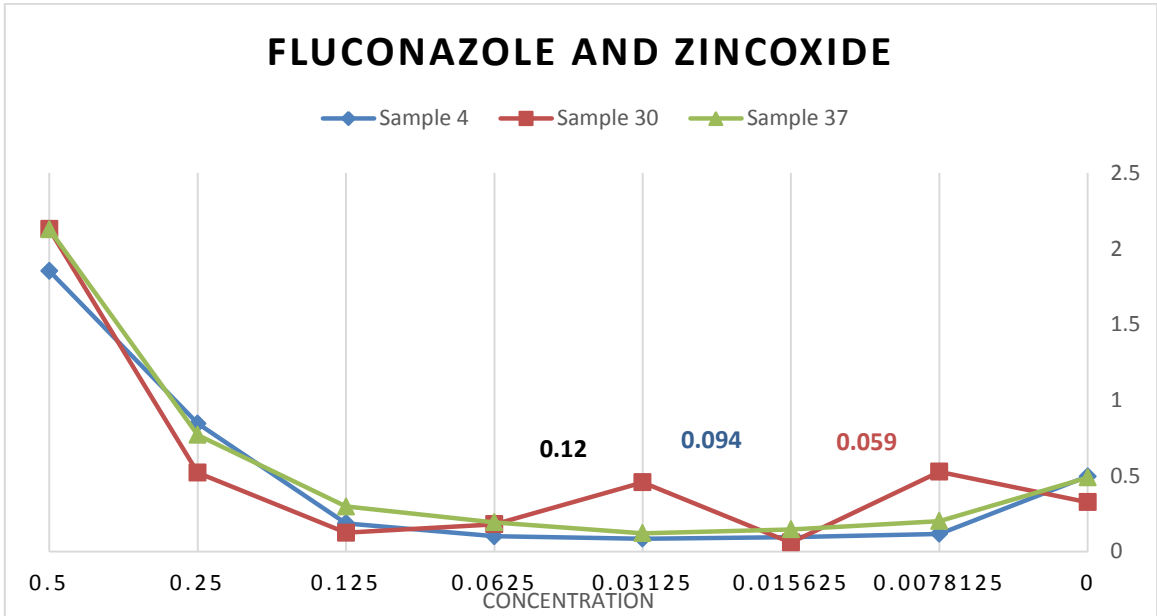


Chart 3: Determination of MIC of Floconazole and Zincoxide in this study

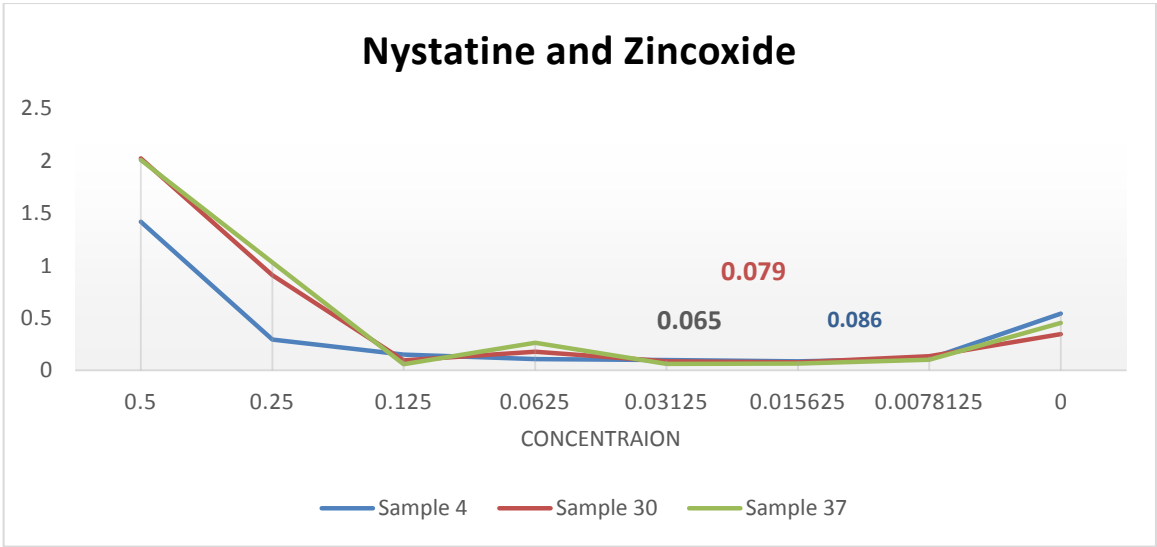


Chart 4: Determination of MIC of Nystatin and Zincoxide in this study

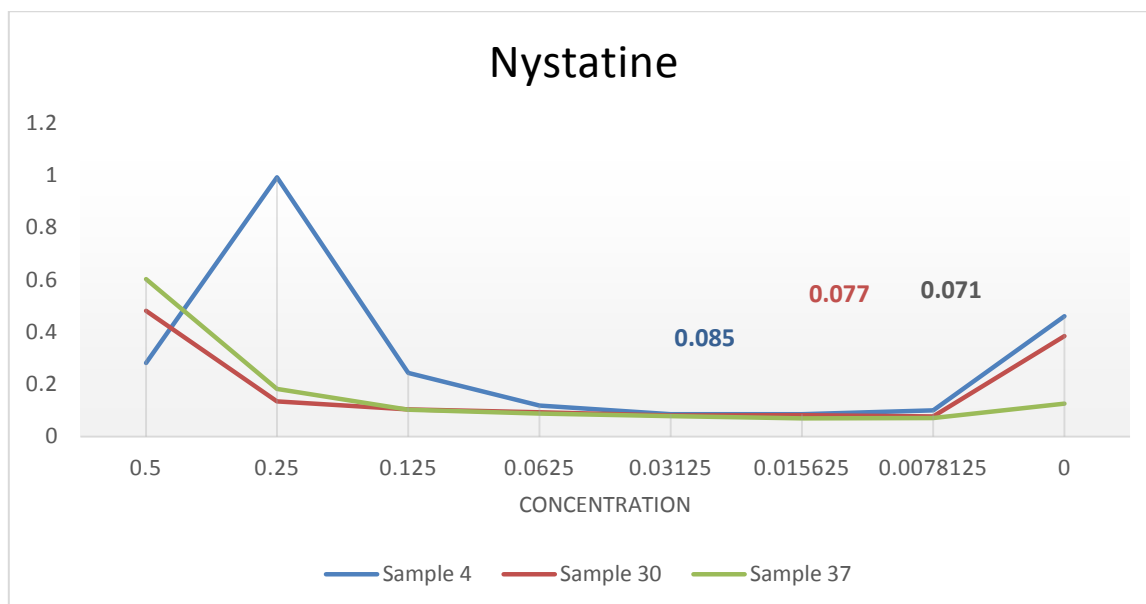


Chart 5: Determination of MIC of Nystatin in this study

3.2 Discussion

Candida spp. is a component of the typical flora of the human skin, mouth, vagina, mucous membranes, and digestive tract (Sopian *et al.* 2016). *Candida albicans* is the major agent of all distinct forms of candidiasis (Filler and Sheppard 2006), especially VVC, which is correlated with both infection and colonization and accounts for 80–90% of all cases (López-Martínez 2010; Mendling and Brasch 2012; Török *et al.*, 2016). All human mucosal surfaces are colonized by *Candida* spp. shortly after birth, and the possibility of endogenous infection is always present (Al-Kobaisi 2007). The incidence of Vulvovaginitis Candidiasis (VVC), an infection of the vulva and vaginal mucosa by yeasts of the genus *Candida* (Criseo, Scordino and Romeo 2015) that causes irritation, pruritus, and vaginal discharge (Al-Kobaisi 2007), has skyrocketed in recent years. The VVC is the leading cause of women seeking gynecological care worldwide (Farage *et al.*, 2010).

In the present investigation, standard and CHROM agar methods yielded comparable findings for the species of *Candida*. However, CHROM agar has the benefit of being speedy, easy, and cost-effective compared to traditional methods, which are sluggish, technically demanding, and expensive (Baradkar *et al.*, 2010; Vijaya *et al.*, 2011). The colonies of *C. albicans* and *C. dubliniensis* appear lighter and darker green, while *C. tropicalis* colonies appear dark blue to metallic blue, as for *C. krusei* colonies, they appear pale pink and dry with a light border

(Abdulla and Ismael, 2022). Other yeasts are white or have a light to dark pink hue (Murray *et al.*, 2005). In this study, *C. albicans* was identified in all samples with positive fungus cultures, equivalent with others (Jamilian *et al.* 2007; Ahmad and Khan 2009; Kennedy and Sobel 2010; Mahmoudabadi *et al.*, 2010; Gumral *et al.* 2011).

In this study, most of the isolated *Candida* spp. from vaginal swabs formed strong biofilm (32.1%), followed by moderated and weak biofilm (10.7%) for each type. However, other *Candida* spp. are also implicated in biofilm-associated illnesses (Iñigo, Pemán and del Pozo 2012). According to (National Institutes of Health) NIH, pathogenic biofilms are responsible for about 80% of all microbial illnesses (Ramage *et al.*, 2006; Perlroth *et al.*, 2007; Tsui *et al.*, 2016; Lohse *et al.*, 2018), more than 75% of all microbial infections in women are vaginal yeast infections, and these infections can occur once or multiple times throughout a woman's life (Yapar 2014).

This disease has been shown a negative effect on life of many women, whereas high rates of females' daily visit clinics according to the survey of this study. The majority of these females have history with RVVC. This is attribute to many reasons, including education level (Felix *et al.* 2020), whereas low education level of these women makes the dealing with the disease more difficult. In addition, the person hygiene is important for avoid the infection, including clean vagina, choice of appropriate washes, change underwear on a daily basis, Maintaining vaginal hygiene for sexual intercourse (Cahyaningtyas 2019). Moreover, there is an effect on the psychological state of women, as a result of the

exhausting symptoms of *Candida*, lack of husband acceptance led to family disintegration (Moshfeghy *et al.* 2020).

The current study also included number of gynecological clinics in Erbil, the cost of daily examination and the cost of medications. Based on the collected data, the total cost per day in all clinics (56,837,550 IQD) and therefore per year is (11,822,210,400 IQD). These costs are considered to be a burden on families and the financial budget for each house. This is in addition to the costs of medication and extra laboratory tests that may be required by the patient. Such burden for a disease that is not considered to be detrimental is of concern, as it will adversely affects the family integration and future perspectives. This study is considered to be the first and unique that shaded light on the burden of *Candida* infection on the life and progress of families in Erbil City, Kurdistan Region of Iraq.

Furthermore, the antifungal agents used have weak or no effect against *Candida* spp., making them difficult to treat. the antifungal agents, which utilized in this study, show weak or no effect against *Candida* spp., this may attribute to that the majority of *Candida* spp. are biofilm producers, which in turn make these organisms more difficult to treat (Saporiti *et al.* 2001). Also, the utilized antifungal agents were varied in quality control based on their origin (Abdulla and Ismael, 2023). It also affects many aspects of women's lives in terms of economy due to the cost of many medications as well as examination by the gynecologist. Frequent visits to the doctor take the time of women.

Nanotechnology is a recent and contemporary branch of science that is one of the most rapidly developing study topics. It focuses on the production of new nanoparticles and nanomaterials with dimensions ranging from 1 to 100 nanometers (Shalaby *et al.* 2015; Hosseini *et al.* 2020). The combination of ZnO-NPs with antifungals used in this study were significantly more effective than when they have been used alone, with a significant correlation between time of resulting ($P < 0.001$).

The results obtained from this research are considered as a tool and information to shed more light on antifungals as well as nanoparticles.

4. Conclusion and future direction

The burden of RVCC is concerned fact as it can cause various effects on the lifestyle, health index and family integration. Additionally, this study

suggests a novel alternative method for treatment of fungal infections. Nanotechnology is a good choice for support the activities of traditional antifungal agents and induce their effects. The present results can pave the way toward seriously introduction of nanotechnology into the medical applications.

Future directions can lead the investigation toward the followings:

- 1- Consideration should be taken for the burden of RVCC on the health index of the females of Kurdistan Region through further thorough investigations.
- 2- More research required for investigate the role of ZnO nanoparticles against *Candida albicans*.
- 3- *In vivo* studying the side effects of these nanoparticles in order to avoid their toxicity effect to human.
- 4- Educational programs by specialists in the field of gynecology to teach women personal hygiene as well as hygiene before and after marital relationship.

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