



Evaluating the Risk of *Candida albicans* Associated with Gestation amongst Women in Port Harcourt, River State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author TS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author APG managed the literature searches and the laboratory analyses of the study, under the supervision of author TS. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPR/2019/v3i430098

Editor(s):

(1) Dr. Ohanu, Ekechukwu Martin, Senior Lecturer, Department of Medical Microbiology, College of Medicine, University of Nigeria, Ituku Ozalla Campus, Enugu, Nigeria.

Reviewers:

(1) Suzanne L. Reiter, USA.
(2) Anslem Ajugwo, Madonna University, Nigeria.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/54597>

Original Research Article

Received 06 December 2019
Accepted 12 February 2020
Published 19 February 2020

ABSTRACT

Candidiasis is increasingly affecting women in developing countries, due to several factors relating to environmental and physiological conditions of the individual. Knowledge of the factors influencing the epidemiology of *Candida* spp. will be useful in formulating public health measures targeted at reducing the incidence of *Candidiasis*. This study was therefore conducted to determine the prevalence of *Candida albicans* amongst women in Port Harcourt, Rivers State, in order to decipher the epidemiology of *Candida albicans* in relation to age and gestation. A total of seventy (140) vaginal swab samples were collected from 70 pregnant and 70 non-pregnant women in Port Harcourt, and analyzed using standard microbiological methods. The result showed that 36% of the total women studied had *Candida albicans* while 64% were negative. In the overall analysis, the distribution of the yeast was 43% prevalent in pregnant and 29% in non-pregnant women. The risk of *Candidiasis* in pregnancy was evaluated using the Odds Ratio (OR) and was determined to be 1.88. Also, a paired sample t-test indicated a positive and strong statistical relationship between age distribution and the presence of *Candida albicans* in women, with a correlation coefficient of

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0.911 (91.1%) ($p < 0.05$), indicating a higher incidence among the older group, albeit lower in age groups close to menopause. The study concluded that the prevalence is higher in pregnant women, with a significant relationship with age. Regular screening of women for *Candida* spp. should be advocated and women should as well avoid conditions that dispose them to genital tract infections.

Keywords: Prevalence; *Candida albicans*; gestation; epidemiology; genital tract infections; odds ratio.

1. INTRODUCTION

Candidiasis is one of the leading infections affecting the female genital track. It is reported that three out of every four women are infected by this fungal genera [1]. Several factors are known to influence the prevalence of *candidiasis*, and includes the use of antibiotics, with broad spectrum activities, gestation, diabetes, HIV/AIDS infections [2,3].

The incidence of fungal infections including infections caused by *Candida* species has increased significantly, and has contributed to high morbidity and mortality rates. *Candida* species have been reported to among the major human fungal pathogens that cause both mucosal and deep tissue infections [4]. *Candida albicans* is the most common species of fungi causing opportunistic infections in humans. They exist as normal flora of the human micro biomes and only become pathogenic when changes in the physiological status and other environmental factors favour their pathogenesis. *Candida albicans* is an oval yeast with a single bud, prevalent in the mouth of infants who are less than a month old, elderly and people with weak immune system; though *Candida* vaginal infections occur more often during pregnancy [5]. Those at the risk of this infection therefore include those suffering from HIV, diabetes, those on antibiotics, pregnant women, cancer and intensive care unit patients [6].

Candida species are not considered as sexually transmitted diseases, as they are seen as normal vaginal flora in healthy women vulvovaginal and as well affects children and celibate women. This does not however, exclude sexual transmission. Cultural isolation and identification has remained crucial in the epidemiology of candidiasis [7]. The samples for such diagnosis is however dependent on region of the body affected. For vaginal candidiasis, swab samples are normally collected from the female and sent to the laboratory for identification.

Researchers have observed an increased susceptibility of the vagina to infection by

Candida species resulting in both a higher rate of vaginal colonization and a higher rate of symptomatic vaginitis [8]. There however, a difference in the susceptibility of the female genital tract to yeast infection due to difference in estrogen level, which enhances adherence of yeast cells to the vaginal mucosa. Estrogen levels and glycogen during child bearing years are said to be higher and will concomitantly cause a higher rate of vaginal candidiasis during pregnancy [8,9]. Jindal et al. [10] and Deorukhkar et al. [11] have reported a high incidence of VVC in patients on oral contraceptives and thus implicates pregnancy control pills as another risk factor associated with vaginal candidiasis, albeit oral contraceptives and pregnancy control pills are not recognized in clinical practice/globally.

If *Candida* is left untreated, it grows worse during pregnancy and may cause complications in pregnancy such as chorioamnitis, abortion, preterm delivery and congenital infection in the neonate. These complications are usually facilitated by conditions that alter normal vaginal flora such as antibiotics, oral contraceptive and contraceptive devices, diabetes etc. [12]. However, on the basis of clinical presentation, microbiology, host factors, and response to therapy, VVC can be classified, according to CDC [13] as either uncomplicated or complicated. The uncomplicated VVC are usually characterized by sporadic or infrequent VVC, mild-to moderate VVC and a diagnosis of *Candida vaginitis* is suggested clinically by the presence of external dysuria and vulvar pruritus, pain, swelling, and redness. Signs include vulvar edema, fissures, excoriations, and thick curdy vaginal discharge. Approximately 10%–20% of women will have complicated VVC, requiring special diagnostic and therapeutic considerations. This may involve recurrent VVC, severe VVC and nonalbicans VVC [13]. According to the US CDC STD document on VVC, women with underlying immunodeficiency, those with poorly controlled diabetes or other immunocompromising conditions (e.g., HIV), pregnant women, and those receiving immunosuppression therapy (e.g., corticosteroid

treatment) do not respond as well to short-term therapies [13]. This eventually accounts for the high prevalence amongst these group.

Several studies have looked at the prevalence of *Candida albicans* in pregnant women [14,15]. However, there is paucity of information regarding the relative risk of *Candida albicans* infection in pregnant women, in relation to the risk in non-pregnant women. The study was therefore conducted with the ultimate aim of determining, comparatively, the impact of age and pregnancy related factors in the causality of vaginal candidiasis.

2. METHODOLOGY

2.1 Study Design

A case-control study design was adopted to investigate the risk of *Candida albicans* amongst women in Port Harcourt, Rivers, Nigeria. It was hypothesized in this study that there was no association between gestation and the occurrence of *Candida albicans* in the female genital tract. The study enrolled 70 pregnant women (the condition of interest) and 70 non pregnant women as control, and analyzed for the presence of *Candida albicans*, to determine the risk during pregnancy. The study covered women of different age group.

2.2 Collection of Samples

High Vaginal Swab (HVS) samples were collected with the aid of a speculum, from subjects attending a tertiary health facility in Port Harcourt, Rivers State, Nigeria, based on informed consent and ethical approval. Samples were transported in an enclosed container to avoid contact with sunlight. Subjects on recent antifungal drugs were excluded in the study.

2.3 Sample Analysis

2.3.1 Microscopy: Wet mount preparation

A saline wet mount preparation of the sample was done for direct microscopy by making a smear of each of the swab sticks on clean grease free glass slides. The slides were viewed under the microscope using x 10 and x 40 objective lens (for multiple pseudohyphae) and the results recorded.

2.3.2 Culture based identification

The samples were cultured, according standard microbiological procedures, on sterile Sabouraud

Dextrose Agar (SDA). A broad-spectrum antibiotics (chloramphenicol) was incorporated to inhibit the growth of bacteria. The SDA plates were incubated aerobically at 37°C for 18–48 h.

Isolates on the SDA plates were identified and speciated using conventional methods. This was achieved by probing the phenotypic attributes on the culture plates alongside the microscopy of the yeast (following staining with a lacto phenol cotton blue stain). The staining was carried out by making a smear of each isolate on a slide, and 2 drops of lactophenol cotton blue stain added. The slides were then viewed under the microscope using x 40 objective lens, and the results recorded.

2.3.3 Germ tube test

Blood sample of a patient was taken and 0.5 ml of serum was used in inoculating the suspected *Candida albicans* spp. into the serum and incubated at 37°C for 2 – 4 hours, which was later viewed under the microscope by putting a drop on a clean glass slide and viewed using x 40 objective lens. Positive results showed germ tube appearance.

2.4 Statistical Analysis

The statistical analysis applied included percentage frequency (% *f*), given by:

$$\% f = \frac{x}{n} \times 100$$

Where *x* is an item and *n* is the frequency of the sample.

However, to determine any significant relationship, the paired sample t-test was used at 95% confidence interval.

3. RESULTS AND DISCUSSION

3.1 Prevalence of *Candida albicans* in the Women Population Studied

A total number of 140 women were sampled for this study that comprised of 70 pregnant women and 70 non pregnant women. The analysis revealed that 50 women representing 36% of the population were positive for *Candida albicans*, while 90 women representing 64% of the population were negative (Fig. 1). This is similar to the work of Akinbiyi et al. [15] on the prevalence of *Candida albicans* among women attending Federal Medical Center Asaba, South-South, Nigeria. In their study of 400 samples, the

overall prevalence of *Candida albicans* was 137 (34.25%).

3.2 Comparative Analysis of Candidiasis in Pregnant and Non-pregnant Women

This comparison was carried out to decipher the risk ratio of *Candida albicans* during pregnancy. The analysis however, revealed that 30 pregnant women representing 43% of pregnant women's population were positive for the yeast (Table 1) while 20 non-pregnant women representing 29% of non-pregnant women's population were infected (Table 1). This shows that 57% of pregnant women's population was infected, while 71% of the non-pregnant women's population was not infected. The result of this investigation is in line with the work of [9] on prevalence of vaginal candidiasis among pregnant women in Nnewi Town of Anambra State, Nigeria. In the study, Ninety patients were positive for vaginal candidiasis thus, giving a prevalence rate of 30% having the highest occurrence.

The prevalence (43%) among pregnant women in Port Harcourt observed in this study is similar to the 40% reported by Uzoh et al. [16]. The prevalence of candidiasis among non-pregnant women (29%) reported in this work [16] was however discordant. This variation may be due to several factors, including the age group and physiological conditions of the subjects.

3.3 Evaluating the Risk of Candidiasis in Pregnancy Using the Odds Ratio (OR)

From Table 1, the odds ratio, also called the cross-product ratio was determined as follows;

$$OR = \frac{a \times d}{b \times c} = (30 \times 50) / (40 \times 20) = 1.88$$

The confidence interval (CI) was estimated using the log of odds ratio, $\log(or) = \log(a \cdot d / b \cdot c)$, and calculate its standard error (se):

$$se(\log(or)) = \sqrt{1/a + 1/b + 1/c + 1/d}$$

The confidence interval, ci, is calculated as:

$$CI = \exp(\log(or) \pm Z_{\alpha/2} \cdot \sqrt{1/a + 1/b + 1/c + 1/d});$$

Where $Z_{\alpha/2}$ is the critical value of the Normal distribution at $\alpha/2$ (using a confidence level of 95%, α is 0.05 and the critical value was 1.96).

The CI was therefore computed to be 0.93, 3.78.

The odds ratio provides a reasonable approximation of the risk ratio, and from this study, the OR is greater than 1, which indicates a reliable association between pregnancy and candidiasis in the population studied and also shows that the risk is generally higher amongst the pregnant group.

Calculating a confidence interval gives an indication of how reliable the odds ratio is; the wider the interval, the greater the uncertainty associated with the estimate. At 95% confidence level, it would be expected that the true value lies within these range of 0.93 and 3.78. This range is narrow and indicates that the odds ratio estimated in this study are reliable, as wider intervals are more uncertain.

Odds ratio has been used by previous researchers [3] to evaluate the risk factors for candida infection of the genital tract and observed that pregnancy was associated with an increased vaginal colonization rate and stimulate the adherence of *C. albicans* to vaginal epithelia cells. In their study, the adjusted OR was determined to range between 1.08 and 2.05, which was consistent with the findings of this work.

In the review of Martins et al. [17], during pregnancy *Candida* spp. colonization increases from 30-40% thus increasing the prevalence of candidiasis among pregnant women. This has been confirmed in this study as the prevalence was found to be higher in the pregnant group.

Similar prevalent rate among pregnant women has been reported from Northern Nigeria [18,19]. Thus, in pregnancy, VVC can be prolonged and associated with more severe symptoms, and resolution of symptoms will typically require longer courses of therapy.

The age group of 21-30 years represents the peak of childbearing in the Nigerian society; and it is among this group that significantly high prevalence of candidiasis occurs. It has been observed that there is high concentration of estrogen hormone during pregnancy and this provides favourable environment for the growth of *Candida*. Advance in age on the other hand, reduces the effect of the estrogen hormone in women, which could lead to lower infection rate as women advance in age. However, no age group was absolutely free of infection by *Candida* sp.

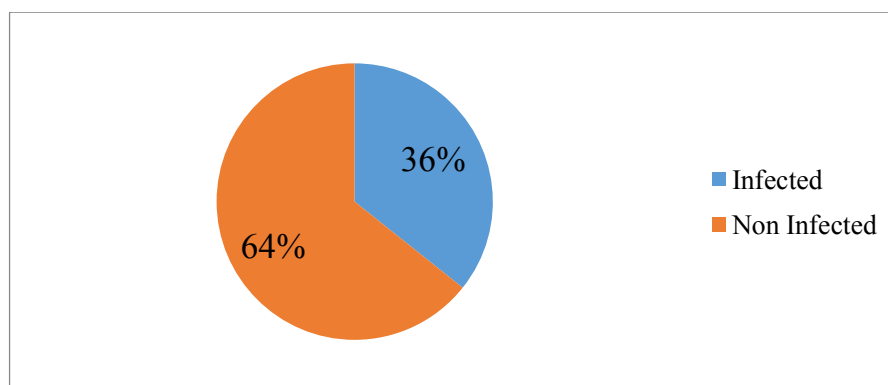


Fig. 1. Overall incidence of genital tract candidiasis in the women studied

Table 1. Incidence of *Candida albicans* in pregnant and non-pregnant women

Subjects	Total number	No. infected	No. not infected	Incidence rate (%)
Pregnant	70	30 (a)	40 (b)	43
Non-Pregnant	70	20 (c)	50 (d)	29

Table 2. Distribution for cases of *Candida albicans* infection

S/N	Age range (years)	Number of women	Infected (%)	Non-infected (%)
1	Below 20	4	2 (50)	2 (50)
2	21-25	42	18 (42.9)	24 (57.1)
3	26-30	40	12 (30)	28 (70)
4	31-35	36	16 (44.4)	20 (55.6)
5	36 and above	18	2 (11.1)	16 (88.9)
Total		140	50	90

3.4 Age Specific Rate of Candidiasis in the Study Group

The age-specific frequency of *Candida albicans* in the group is as shown in Table 2. The result shows that rate was higher within the active child bearing age and was least at age groups close to menopause (36 years and above). This could also be as a result of sexual activity within the age groups, as sexual activity and philandering tends to be more within these age groups. The result of the study is in line with the study of [7] as well as [16]. In the study of Uzoh et al. [16], the age 20-29 had the highest prevalence of *Candida albicans* with 77(38.5%), followed by ages 30-39 with prevalence of 50 (33%) and the least was ages 40-49 with prevalence of 2.

In this study, the age range of women of 36 years and above showed the least prevalence of candidiasis. This is also in line with the study of Okonkwo [9] who reported that the number of older pregnant women (age group 41-45) recorded in the study was small. Thus it can be because they may be passing the childbearing

age and women tend towards menopause (cessation of menstruation) as age increases and only few may conceive within this age group.

A paired sample t-test was conducted to determine if there was a significant relationship between age distribution and number of infected women at 95% confidence interval of the difference. The t-test revealed a statistically reliable relationship between age distribution of women and number of infected women. The correlation coefficient was 0.911 (91.1%) indicating a very strong and positive correlation between age distribution and number of infected women ($\rho < 0.05$). This means that the older the women get, the more chances of candidiasis. This increase in the chances of candida infection with increase in age may be connected with the reduction in the immune system. Also, in the study of Pfaller and Diekema [20], age was a factor related to the infection. In their univariate analysis, the average ages in cases was generally older than controls (35 years versus 32 years, $p=0.003$), which was consistent with previous study. Garcia et al. [21] observed that

the incidence of *Candida* infection increased with age.

These observations are however different from the work of Anthony et al. [7] on the prevalence of vulvovaginal candidiasis (VVC) among non-pregnant women attending a tertiary health care facility in Abuja, Nigeria. In their study of 200 participants as subject, there was no significant relationship between the prevalence of VVC with age distribution or other prevailing health conditions studied ($p > 0.05$). This difference is attributable to other factors relating to host conditions.

4. CONCLUSION

Candidiasis is one of the prevalent diseases among women in Port Harcourt. A relatively high prevalence of *Candidiasis* was observed in this study. This research therefore concludes that candidiasis is a common disease common among women, both pregnant and non-pregnant albeit higher during conception. The study has also shown that the prevalence varied according to age group with a decreasing incidence amongst women close to menopause. This therefore shows that age and gestation influences the epidemiology of candidiasis in women. It follows that the younger groups are more predisposed to conditions that favour the colonization of the female genital tract by *Candida* spp.

The odds of candidiasis in pregnancy were estimated to be 1.88 times the odds of candidiasis in the non-pregnant group. This is therefore a confirmation that the risk of candidiasis is higher during pregnancy. At 95 % confidence level it is reliable to infer that the odds of candidiasis in pregnancy are quite certain. This therefore implies that the probability of women having candidiasis is higher during conception. Women should therefore avoid conditions that dispose them to candidiasis. Since pregnant women are more prone to this infection, they should be very careful during their period of gestation. Regular screening of women for *Candida* spp. should be advocated and women in general should as well avoid conditions that dispose them to genital tract infections.

We also recommend that research relating to the epidemiology of candidiasis, to pry into the risk factors that influence the outcome of the investigation should consider the age specific

rate and should be standardized when comparing the incidence in different locations/population.

CONSENT AND ETHICAL APPROVAL

As per international standard guideline participant consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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