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Evaluation of Candidiasis among pregnant women attending antenatal clinic in University of Benin teaching hospital (Ubth), Benin city, Edo state

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Abstract

It has been proved that vaginal candidiasis is a common and serious health problem among women of Child bearing age which is linked to Candida. It is more prevalent and serious in women with depressed immunity especially in pregnancy.

High vaginal specimens (HVS) were obtained from 100 pregnant women from age 18-41 attending antenatal clinic of the Tertiary Health Care from April to June 2014 and 20 high vaginal swabs (HVS) were collected from non-pregnant women as control. The swab specimen was examined using Gram stains, wet preparation method, and culture. Germ-Tube test was particularly used for the *Candida albicans*. 58% tested positive for vaginal candidiasis by pregnant women while 40% of non-pregnant women tested positive. Three species of *Candida* were observed to be common in vagina of the woman studied namely *Candida albicans*, *Candida krusei* and *Candida tropicalis* comprising 46.6%, 23.8% and 16.6% respectively. The study showed that pregnant women aged 28-32 years were observed to have the greatest level of vulvovaginal candidiasis, although, it was not identified as a risk factor for acquisition of vaginal candidiasis in this study ($P= 0.224$). The Prevalence of vaginal candidiasis was significantly affected by trimester of pregnancy ($P= 0.0004$). Pregnant women in the third trimester had the highest risk of acquiring vulvovaginal candidiasis. The Health workers handling pregnant women should enquire about symptoms of vaginal candidiasis to ensure definite diagnosis and treatment and enlighten them on the preventing measures.

Keywords: Candidiasis, Pregnant women, Antenatal Clinic

Introduction

It is reported that vaginal candidiasis (VC) is a fungal infection of the Vulva and/or vagina. It is a frequent gynecologic disease, affecting 3 out of 4 women in their life (Das-Neves et al., 2008). Vaginal candidiasis shows to present most times in the area of raised estrogen levels. It is observed to be higher than 40% of affected women would have 2 or more VC episodes (Ferrer, 2000; Eschenbach, 2004). It has been recorded in United States that the most dominant yeast infection

is candida vulvovaginitis (Sobel, 1992). It is reported that at least 75% of all women suffer from at least one attack of candida vulvovaginitis during their lifetime and nearly half of them suffer many times (Saporiti et al., 2001; Ferrer, 2000). In about 5% of cases of candida vulvovaginitis, the disease has a chronic manifestation (Ferrer, 2000). Based on the age, locality and social economic status; prevalence of vaginal yeast isolates has been shown to be between 5 and

48.4% in women (Abu-Elteen *et al.*, 1997). A study conducted in Nigeria by Enweani *et al.* (2001) reported that the prevalence of candida vulvovaginitis was 40.6%.

Women who have candida vulvovaginitis experience symptoms. The itching can be either external or internal. This causes high discomfort and disturbs the wellbeing of women. It could also make walking, urinating or having sex very painful. This case can occasionally manifest in the healthiest of women. However, it is more prevalent and serious in women with immunosuppression which pregnancy is one the factors (Monif and Baker, 2003).

Establishing Candida as the agent of vaginitis can be a great task as up to 50% of asymptomatic women do have Candida organisms as part of their endogenous vaginal flora; hence limitations of signs and symptoms in the diagnosis of vaginal infection has been seen (Akinbiyi, Robert and Paul, 2009). Thus, mere isolation of Candida in the laboratory does not reveal real indication that it is the cause of any disease condition as other causes of vaginitis may include *Trichomonas vaginalis* and bacterial vaginosis.

Under normal circumstances, the Candida yeast is held in check by normal body defenses together with other members of the normal flora. For instance, the acidity of the vagina is maintained at pH 4.0-4.5 (Nyirjesy, 2008). This acidity prevents some vaginal pathogens from establishing. However, physiological in the balance of the body system would affect both beneficial and casts, bacteria and other organisms in the body. This accordingly would Alter the acidity of the vagina reducing it to pH 5.0-6.5, thereby giving room for the establishment of pathogenic organisms such as candida (Akinbiyi *et al.*, 2008).

Vaginal pH may increase with age, phase of menstrual cycle, sexual activity. Contraception choice, pregnancy, presence of necrotic tissue or foreign bodies, and use of hygienic products or antibiotics (Nyirjesy, 2008).

It is generally believed that increased estrogen degrees and higher glycogen content in vaginal secretions during pregnancy increase a woman's risk of developing VC, and it is regarded to be so common in women during their child-bearing years (Monif *et al.*, 2003). The presence of Candida in urine is referred to as candiduria. The majority of patients with candiduria suffer a completely benign process (Kauffman *et al.*,

2000). However, candiduria is sometimes a marker of disseminated candidiasis (Nassoura *et al.*, 1993). Oro-pharyngeal candidiasis is considered to be the most common opportunistic fungal disease in HIV/ AIDS patients globally, occurring in an estimated 80-95% of those with HIV disease (Priscilla *et al.*, 2002; Hodgson *et al.*, 2002). *Candida albicans* is the most common yeast implicated in oro-pharyngeal candidiasis (Ebrahim *et al.*, 2002) and (Dunic *et al.*, 2004) have showed prevalence levels of 52.4% and 77,700 respectively from HIV/ AIDS patients. *C. albicans* infection happens in the vast majority (80% to 90%) of diagnosed cases, while infection with other species. Such as *C. glabrata* or *C. tropicalis* occur less frequently (Baron *et al.*, 1993). Since there are no vaccines currently licensed for preventing yeast infections, the only clinical recourse to combat yeast infections is the use of antifungal agents. Antifungal agents commonly used to treat candida vulvovaginitis are topical clotrimazole, topical nystatin, fluconazole and topical ketoconazole. Other antifungal agents such as amphotericin B, voriconazole, flucytosine and capsosungin are used in treating systemic yeast infections. With adequate pharmacotherapy and avoidance of contributing factors (eg, douching, wearing tight pants), VC and associated symptoms resolve in a short period of time

Aim

The study was done to determine the prevalence of vaginal candidiasis among pregnant women attending antenatal clinic in University of Benin Teaching Hospital (UBTH).

Specific Objectives

1. To determine the prevalence of vaginal candidiasis in clinically symptomatic and asymptomatic cases of pregnant women attending routine antenatal clinic in University of Benin Teaching Hospital (UBTH) in Edo State.
2. To determine the age-related infection with candidiasis in the pregnant women
3. To determine the prevalence of vulvovaginal candidiasis among pregnant and non-pregnant women in this research study.
4. To determine if the prevalence of vaginal candidiasis is significantly affected by the trimester.

Materials and Methods

Study area

The study was carried out among pregnant women attending the ante-natal clinic of the University of Benin Teaching Hospital (UBTH) of Credo Local Government area of Edo State

Criteria for subject selection

All subjects were pregnant women within the ages of 18-41 years attending antenatal clinic in the hospital aforementioned and were in different trimesters of pregnancy. Simple questionnaire was used in getting requisite demographic data about the patients. All the pregnant women who complained of certain symptoms such as vaginal discharge, vulval itching, painful sensation after urination and others were regarded symptomatic and others classified as asymptomatic.

Exclusion criteria

All male subjects were not enrolled in the study. Also, those who did not give their consent were excluded from the study.

Ethical clearance and consent

The study was approved by the ethical committee of the University of Benin Teaching Hospital (UBTH). The study was first introduced to the pregnant women on their visit to the antenatal clinic in order to obtain their consent. The procedure and purpose of the study was also explained to the pregnant women before their samples were collected.

Study design

The research was done from April-June, 2014. A consent form was shown to all the subjects and the high vaginal swab (HVS) samples were collected from those who gave their consent. The collection was done during visitation by the pregnant women to the antenatal clinic, Gram stain, microscopy, culture on Sabouraud Dextrose agar (SDA) and germ tube test were carried out on the samples.

Study population

A total of 120 women consisting of 100 pregnant and 20 non-pregnant women presenting with signs and symptoms of vaginitis from the University of Benin

Teaching Hospital Benin City were selected for the study. Participants' ages ranged from 18-41 years.

Collection and processing of samples

From each subject, two High vaginal swabs (HVS) were collected by attending physician, To do this sterile cotton wool swab was inserted carefully into the upper part of vagina with the aid of speculum and rotated gently before withdrawing. The swab was inserted into a tube from which it was taken. The tube containing the swab was labeled with the patients name, age and sex and then transported to the laboratory. In the laboratory the swabs were given laboratory identification numbers and were processed immediately.

Gram stain

Smears from the first vaginal swabs were prepared on slides cleaned with alcohol. They were then heat-fixed. Staining was done by flooding the smears with crystal violet solution for 1 min and then with Gram's iodine for 1 min and washing with water in between application of stains. Smear was decolorized briskly with acetone, and counterstained with neutral red for 2 minutes. Slide was washed with water and allowed to dry, The slide was subjected to observation of Candida morphology under oil immersion objective lens (100X) of a Bright Field microscope.

Wet preparation

The method described by Cheesbrough (2000) was applied. 1 ml of physiological saline was added to first swab and a smear subsequently made on a clean grease free slide and covered with a cover slip, The preparation was examined under the microscope using the X 40 objective lens for the presence of pseudohyphae and/or budding yeast cells indicative of Candida

Culture of specimen

The second swab was inoculated onto Sabouraud dextrose agar (Difco) and incubated for 24-96 h at 37°C in ambient air atmosphere. Agar slants were read and observed for the emergence of white to cream coloured, smooth and glabrous colonies which were regarded as suspect Candida species.

Germ tube test

Germ tube test was carried out specifically for the identification of *Candida albicans* as previously described by Nelson *et al.* (2013). Briefly a single colony of the test yeast cells from a pure culture was inoculated in human serum and incubated at 37°C for 2 -4 hours. A drop of the incubated serum was placed on a microscope slide and covered with a cover slip. The wet mounts were examined under the microscope for the presence of germ tube using the 40X objective (Dalmau morphology method). The isolate were classified as either germ tube positive or germ tube negative.

Chromogenic speciation of candida isolate on chromagar candida

Using already prepared CHROMAGAR Candida plates, the Candida isolates were streaked carefully and incubated for 48 hours before they were read according to manufacturer's instruction. CHROM agar Candida is a differential medium for the isolation and presumptive identification of clinical important yeasts within 24 and 48 hours on the basis of strongly contrasting colony colours. As such, the light to medium green colonies were identified as *Candida albicans*, steel blue colonies accompanied by purple pigmentation diffused into surrounding agar as *Candida tropicalis* and *Candida krusei* by growth as large, fuzzy, rose coloured colonies with white edges.

Statistical analysis

The data obtained were analyzed using Percentage prevalence. Statistical package for Social Sciences (SPSS version 16 using Chi square (X^2) test. Statistical significance was set at $P < 0.05$.

Results

The prevalence of vulvovaginal candidiasis among pregnant and non-pregnant women in this study was 58.0% and 40.0% respectively. Although pregnant women were observed to have a higher prevalence of vulvovaginal candidiasis than not pregnant women, the difference did not reach statistical significance (Pregnant vs non pregnant 58.0% vs. 40.0%: OR: 2.074, 95% CI =0.778, 5.514, $P = 0.149$) (Table 1).

Pregnant women within the age group of 28-32 years were observed to have the highest prevalence of vulvovaginal candidiasis. Age however was not identified as a risk factor for vulvovaginal candidiasis among pregnant women ($P=0.224$) (Table 2)

The prevalence of vulvovaginal candidiasis was significantly affected by trimester of pregnancy ($P = 0.0004$). Pregnant women in the third trimester had the highest risk of acquiring vulvovaginal candidiasis (Table 3).

Table 1: Prevalence of vulvovaginal candidiasis among pregnant and non-pregnant Women

Characteristics	N	No Pos (%)	OR	95% CI	P value
Pregnancy Status					
Pregnant	100	58 (58.0)	2.074	0.778, 5.514	0.149
Non Pregnant	20	8 (40.0)			

N - Number of participants; OR - odd ratio; CI - confidence interval

Table 2: Effect of age on prevalence of Vulvovaginal candidate among pregnant women

Characteristic	N	No Pos (%)	P value
Age (Years)			
18-22	25	14(56.0)	0.224
23-27	28	19(67.9)	
28-32	19	13(68.4)	
33-37	20	9(45.0)	
≥ 38	8	3(37.5)	

N- Number of participants

Table 3: Effect of trimester on prevalence of Vulvovaginal candida among pregnant women

Characteristic	N	No Pos (%)	P value
Trimester			
1 st	26	10(38.7)	0.0004*
2 nd	32	14(43.8)	
3 rd	42	34(80.9)	

N- Number of participants *-significant

Table 4 -Comparison of candida species in subject and control

AGE GROUP	Subject with <i>Candida albicans</i>	Subject with <i>Candida krusei</i>	Subject with <i>Candida tropicalis</i>	Total
18-22	6	4	4	14
23-27	10	5	4	19
28-32	5	5	3	13
33-37	4	3	2	9
38	2	1	0	3

P value -0.224

Discussion

Out of the 100 pregnant women examined, 58 (58%) were positive. This is higher than 40.0%, 44.8%, 55.2% reported by Nwadioha *et al.* (2010), Oviasogie and Okungbowa (2009), Nwokedi and Anyim, 2003 respectively. It is however higher than 55.4% and 14% reported by Taou *et al.* (2013) and Meda *et al.* (1997) respectively. The observed variation could be as a result of differences in location, living hygienic standards and immunity of pregnant women in these studies. In this study vulvovaginal candidiasis was recorded among 8 non pregnant women representing 40.0% of women examined. This figure is lower than 44.8%, 16.7%, 46.7% and 35.4% recorded by Dias *et al.* (2011), Umeh and Emelugo (2007), Oviasogie and Okungbowa (2009) and Toua *et al.* (2013) respectively.

Although pregnant women were observed to have a higher prevalence of vulvovaginal candidiasis than non-pregnant women, the difference was not statistically significant; this is in disagreement with an earlier study by Toua *et al.* (2013). Pregnant women are ordinarily expected to have a significantly higher prevalence of vulvovaginal candidiasis than non-pregnant women since reports show that higher oestrogen levels and glycogen content in vaginal secretions during pregnancy increases a woman's risk

for developing vulvovaginal candidiasis (Okonkwo and Umeanaeto, 2010), but this was not the case in this study with about 58% positive for vaginal candidiasis in pregnant women and 40% positive in non-pregnant women and the difference not statistically significant.

Age of pregnant women was not identified as a risk factor for acquisition of vulvovaginal candidiasis in this study ($P = 0.224$). This is consistent with findings from an earlier study by Oviasogie and Okungbowa (2009). The highest prevalence of vulvovaginal candidiasis was recorded among pregnant women within the age group of 28-32 years. This agrees with the reports of other researchers such as Toua *et al.* (2013), Oviasogie and Okungbowa (2009). The set of women with the highest risk for vulvovaginal candidiasis in this study falls within the age range for child bearing women in Nigeria. This may therefore, explain the observed high prevalence of vulvovaginal candidiasis among them.

The prevalence of vulvovaginal candidiasis was significantly affected by trimester of pregnancy ($P = 0.0004$). Pregnant women in the third trimester had the highest risk of acquiring vulvovaginal candidiasis. This is in line with reports of Okpara *et al.*, 2009; Okonkwo *et al.* (2010), Alo *et al.* (2012).

Three (3) species of *Candida* were found in the vaginas of women in Benin, Nigeria with vulvovaginal candidiasis namely-*Candida albicans*, *Candida krusei* and *Candida tropicalis* with *Candida albicans* being predominant representing 46.6%, 23.8% and 16.6% respectively. This is very important because in many Clinical settings in Nigeria, laboratory reports have *Candida albicans* as the only species isolated in women with vulvovagina candidiasis meanwhile others exist. This may explain the reason they are poorly managed with antimycotic regimen. The higher prevalence of *Candida albicans* than other non-*albicans* species may be related to the presence of more secreted aspartic proteinase 5 and 9 in *Candida albicans* (SAP 5 and SAP 9), which are known virulence factors in *Candida* species Dirisu and Agbakoba (2016).

Conclusion

In conclusion, the prevalence of vulvovaginal candidiasis among pregnant women was 58.0% and was higher than 40.0% recorded among non-pregnant Women, albeit the difference was not statistically significant. Pregnant women within the age group of 28- 32 years were observed to have the highest prevalence of vulvovaginal candidiasis. Age however was not identified as a risk factor for acquiring vulvovaginal candidiasis among pregnant Women in this study. Pregnant Women in the third trimester were observed to have a significantly higher prevalence of vulvovaginal candidiasis than those in first and second trimester of pregnancy.

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