Prevalence of Candida Species among Vaginitis Symptomatic Pregnant Women Attending Ante-natal Clinic of Anambra State University Teaching Hospital, Awka, Nigeria

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Abstract Vaginitis is the fungal infection of the vulva and or vagina caused mostly by *Candida albicans*. Itching, abortion, irritation of the vulva and vagina and white discharges from the vagina are the common symptoms. Thirty vaginitis symptomatic pregnant women attending ante-natal clinic of Anambra State University Teaching Hospital Awka, Nigeria were sampled. They were aged between twenty and forty-five years. The *Candida* species were isolated, characterized and identified by standard biochemical and microbiological methods. The women aged 26-30 years had the highest percentage distribution of *Candida* species (63.3%) while those in their third trimester of pregnancy were mostly infected, with a percentage distribution of 63.3%. The vaginal *Candida* species isolated from the women were *Candida albicans* (66.7%), *Candida grabrata* (23.3%), *Candida tropicalis* (6.7%) and *Candida krusei* (3.3%). *Candida albicans* occurred in all the age groups and trimesters of pregnancy. The pregnant women at great risk of vaginitis are those aged between 26 and 30 years as well as those in their third trimester of pregnancy. This calls for commitment to routine medical examination and appropriate treatment of the infected pregnant women.

Keywords Prevalence, Vaginitis, Pregnant Women, Teaching Hospital, Ante-natal Clinic

1. Introduction

Vaginitis is the infection of the vulva and or vagina by any species of the yeast *Candida*, the most common being *Candida albicans* [1]. Approximately 75% of all pregnant women experience at least one episode of vaginitis during their life time and 50% of them suffer multiple episodes [2]. The infection can be acute, chronic, superficial or deep and

has wide clinical spectrum. *Candida* species are almost universal in low numbers on healthy adult skin and are part of the normal flora of the mucous membrane of the respiratory, gastrointestinal and female genital tracts. Overgrowth of these organisms can cause superficial infections such as vaginitis.

The incidence of vaginitis is more remarkable among pregnant women. Several factors can be associated with the increased role of vagina women colonization by *Candida* species. These include compromised immune system, obesity, diabetes, prolonged use of antimicrobials, HIV/AIDS, pregnancy, poor dietary habits, use of high level of estrogens and oral contraceptives and poor personal hygiene [3-6]. Poorly supported risk factors include the use of sponge, intrauterine devices, diaphragms, condoms, sexual intercourse and diet with high glucose content [7,8].

Vaginitis among pregnant women has also been related to emotional stress and suppression of immune system [9]. During pregnancy, levels of both progesterones and estrogens are elevated. Progesterone has suppressive effects on the anti-candida activity of neutrophils [10] while estrogen has been found to reduce the ability of the vaginal epithelial cells to inhibit the growth of *Candida albicans* and also decrease immunoglobulins in vaginal secretions resulting in increased vulnerability of pregnant women to vaginitis [11]. Premature neonates are severely endangered because of their immature immune system. During delivery, transmission can occur from the vagina of the infected mother to the newborn, giving rise to congenital infection [12]. This study was therefore undertaken to determine the prevalence of *Candida* species among vaginitis symptomatic pregnant women attending ante-natal clinic of Anambra State University Teaching Hospital Awka, Nigeria with a view to creating more awareness about this preventable and treatable infection.

2. Materials & Methods

2.1. Study Design

Thirty pregnant women attending ante-natal clinic of Anambra State University Teaching Hospital Awka, Nigeria were sampled. Information regarding their ages and trimester of pregnancy was obtained through the questionnaires completed by them. Their consent was also obtained before the samples were collected and their ages ranged between twenty and forty-five years. The women sampled had symptoms of vaginitis.

2.2. Sample Collection and Processing

Thirty vaginal swabs were collected from the vaginitis symptomatic pregnant women. The pregnant women that had itching, dysuria, white discharges from the vagina as well as difficulty in walking were deemed symptomatic. Vaginal swab samples were collected from ten pregnant women per day to allow for their processing on the same day. The samples were collected within the period of August, 2014.

A sterile cotton-wool swab stick was inserted into the endocervical canal and gently rotated against the wall of the endocervix. The swab stick was then withdrawn and replaced in its casing and labeled appropriately with the patient's information. The women were placed according to their ages into the following age groups: 20-25, 26-30, 31-35, 36-40 and 41-45 years. They were also stratified according to the trimester of pregnancy into first, second and third trimesters.

3. Laboratory Analysis

3.1. Gram Stain

Smears were made from the vaginal swabs, stained using the gram stain procedure and examined under the microscope for the presence of gram positive budding yeast cells with pseudohyphae.

3.2. Culture Procedure

Each of the vaginal swabs was aseptically inoculated on sabouraud dextrose agar (SDA) supplemented with two percent chloramphenicol to inhibit bacterial growth. The inoculated plates were incubated at 37°C for 48 hours and examined for cream coloured colonies and budding yeast cells. The colonies were purified by repeated subculturing on sterile SDA plates and later stored on sterile SDA slants for further tests.

3.3. Chlamydospores Formation Test

The method of Menza et al [13] was used. The isolates (48

hour old yeast colonies) were inoculated by streaking in corn meal agar containing tween 80 and incubated at 25°C for 72 hours. The isolates were thereafter stained with lactophenol cotton blue on a slide and examined under the microscope for chlamydospore formation.

3.4. Germ Tube Test

The procedure of Menza et al [13] was used in performing the test. A colony of the test yeast cell was inoculated in human serum and incubated at 37°C for 3 hours. A drop of the incubated serum was placed on a microscope slide and covered with a cover slip and examined under the microscope for the presence of germ tube. This is a presumptive test for the identification of *Candida albicans*.

3.5. Sugar Assimilation Test

The procedure of Menza et al [13] was used in carrying out the test. Carbohydrate impregnated filter paper discs were placed on sterile carbohydrate-free yeast nitrogen base agar contained in petri dishes and incubated at 30°C for 18 hours. The presence of growth around the carbohydrate-free filter paper discs was thereafter observed. The presence of growth in the medium indicated the ability of the isolate to assimilate a sugar. The sugars used were glucose, lactose, sucrose and galactose.

3.6. Motility Test

Hanging drop slides were used. Vaseline was spread with a sterile toothpick on the four corners of a clean coverslip. A drop of the yeast suspension was aseptically placed in the centre of the coverslip with a sterile inoculating loop. The depression slide was lowered, with the concavity facing down, onto the coverslip so that the drop protruded into the centre of the concavity of the slide. The hanging drop slide was turned over and placed on the stage of the microscope so that the drop was over the light hole. The drop was thereafter examined under the microscope.

3.7. Urease Test

The surface of a Urea agar slant was streaked with a portion of the test organism. The cap of the tube was left on loosely and the test tube was incubated at 37°C for 48 hours and observed for colour change from light orange to bright pink which was an indication that the organism produced the enzyme Urease.

4. Results and Discussion

The prevalence of *Candida* species among Vaginitis Symptomatic Pregnant women attending ante-natal clinic of Anambra State University Teaching Hospital Awka, Nigeria

was studied. Thirty pregnant women aged between twenty years and forty-six years who attended the ante-natal clinic during the month of August 2014 were sampled.

The percentage distribution of vaginitis according to the age groups of the pregnant women showed that 10% (n=3) were in the age group of 20-25 years, 63.3% (n=19) in the age group of 26-30 years, 16.7% (n=5) in the age group of 31-35 years, 6.7% (n=2) in the age group of 36-40 years while 3.3% (n=1) was in the age group of 41-45 years (Table 1).

Table 1. Percentage distribution of vaginitis according to the age groups of the pregnant women.

Age group (yrs)	Number of pregnant women sampled	Distribution (%)
20-25	3	10.0
26-30	19	63.3
31-35	5	16.7
36-40	2	6.7
41-45	1	3.3

The high percentage distribution of vaginitis in the age group 26-30 years may be because members of the age group are sexually active and use drugs and contraceptives indiscriminately. The incidence rates of vaginitis among the women within the age group 26-35 years and 20-30 years were 80.0% and 73.3% respectively.

Menza et al [13] however reported an incidence rate of 60% within the age group 26-35 years in Kenya, Sehgal et al [14] reported an incidence rate of 54% within the age bracket 20-30 years in Northern Nigeria while Okungbowa et al [15] and Akortha et al [16] reported incidence rates of 55% and 57% respectively within the age group 26-35 years in Benin City, Edo State, Nigeria. Women in the age group 41-45 years are close to their menopause and are less active sexually. They also rarely use contraceptives to prevent pregnancy and have increased vaginal immunity due to decreased levels of estrogens and corticoids. In this study, the percentage distribution of vaginitis in the women aged 36-45 years was 10.0%. This result is in agreement with Okungbowa et al [15] who reported a prevalence rate of 10% within the age group of 36-45 years and concluded that it was probably due to the possible increase in vaginal immunity.

The percentage distribution of vaginitis according to the trimester of pregnancy showed that 10.0% (n=3) were in the first trimester, 26.7% (n=8) in the second trimester and 63.3% (n=19) were in the third trimester of pregnancy (Table 2).

Table 2. Percentage distribution of vaginitis according to the trimester of pregnancy

Trimester of Pregnancy	Number of pregnant Women sampled	Distribution (%)
First	3	10.0
Second	8	26.7
Third	19	63.3

The high percentage distribution of vaginitis among the pregnant women in their third trimester of pregnancy was because pregnant women in that trimester of pregnancy have suppressed immune system which may be due to emotional stress which increases as child birth approaches, increased levels of estrogens and corticoids which decrease vaginal defense mechanisms, vaginal and pelvic examinations in preparation to delivery which predispose the mother to greater chances of vaginitis and reduction in the hygiene status of some pregnant mothers such as failure to wash their undies and private parts properly due to fatigue or stomach size. Pregnant women in their first and second trimesters of pregnancy have low emotional stress and strong immune system against *Candida* species infections. Sobel et al [9] however reported prevalence rates of 11% and 20% in pregnant women in their first and second trimesters of pregnancy respectively. Olowe et al [17] reported that the prevalence of vulvovaginal candidiasis to be highest in the third trimester of pregnancy as virtually all the women they examined had candidiasis compared with 37.5% of those in their second and 25% of first trimester pregnancies.

The percentage occurrence of vaginal *Candida* species in the pregnant women showed that 66.7% (n=20) were *Candida albicans*, 23.3% (n=7) were *Candida glabrata*, 6.7% (n=2) were *Candida tropicalis* while 3.3% (n=1) was *Candida krusei* (Table 3).

Table 3. Percentage occurrence of vaginal *Candida* species in the pregnant women

Vaginal candida species	Number isolated	Occurrence (%)
<i>Candida albicans</i>	20	66.7
<i>Candida glabrata</i>	7	23.3
<i>Candida tropicalis</i>	2	6.7
<i>Candida krusei</i>	1	3.3

Candida albicans was the most common vaginal *Candida* species isolated in this study. This may be as a result of the predominance of the yeast over the other species. This result is in agreement with Akortha et al [16] and Abu-Elteen et al [18] who reported *Candida albicans* as the most common vaginal *Candida* species isolated in the pregnant women they studied. The percentage occurrence of non-*Candida albicans* in this study was 33.3% indicating that they are becoming significant pathogens.

The distribution of the vaginal *Candida* species according to the age groups of the pregnant women showed that all the vaginal *Candida* species were isolated from the pregnant women within the age group of 26-30 years. *Candida albicans* occurred in all the age groups studied while *C. tropicalis* and *C. krusei* were only isolated from women within the age group of 26-30 years and *C. glabrata* in the age group of 20-25 and 26-30 years (Table 4).

Table 4. Distribution of the vaginal Candida species according to the age groups of the pregnant women.

Vaginal candida species	20-25years (n)	26-30years (n)	31-35 years (n)	36-40 years (n)	41-45 years (n)
Candida albicans	2	13	3	2	1
Candida glabrata	1	3	2	0	0
Candida tropicalis	0	2	0	0	0
Candida krusei	0	1	0	0	0

n= number of isolates

The distribution of the vaginal Candida species was highest within the age group of 26-30 years (63.3%). *Candida albicans* had the highest distribution of 66.7% within this age group. The distribution of vaginal Candida species within the age group of 20-30 years was 73.3%. Sehgal [14] however reported a 54% distribution of vaginal Candida species within the age group of 20-30 years in Northern Nigeria. The distribution of vaginal Candida species within the age group of 41-45 years was low (3.3%).

The high distribution of the vaginal Candida species within the age group of 26-30 years may be because women within this age group are sexually active, abuse antibiotics and use contraceptives to prevent pregnancy thereby reducing their vaginal immunity while the low distribution within the age group of 41-45 years was because of increased vaginal immunity occasioned by the fact that the women within the age group are less sexually active and rarely abuse antibiotics. They also rarely use contraceptives to prevent pregnancy. These results agreed with those of Akortha et al [16].

The distribution of vaginal Candida species according to the trimester of pregnancy showed that 10.0% (n=3) were isolated in the first, 26.7% (n=8) in the second and 63.3% (n=19) in the third trimesters of pregnancy (Table 5).

Table 5. Distribution of vaginal Candida species according to trimester of pregnancy

Trimester of pregnancy	Candida albicans (n)	Candida glabrata (n)	Candida tropicalis (n)	Candida krusei (n)
First	3	0	0	0
Second	5	2	1	0
Third	12	4	2	1

n= number of isolates

Candida albicans was isolated from the pregnant women in all the three trimesters of pregnancy because it is the most common vaginal species causing candidiasis. *Candida glabrata* and *C. tropicalis* were isolated in the second and third trimesters of pregnancy only while *C. krusei* was isolated in the third trimester of pregnancy only.

The highest number of vaginal Candida species (63.3%) was isolated during the third trimester of pregnancy. This result is in agreement with that of Sobel et al [19] who

isolated *Candida* species at a rate of 67% in the third trimester of pregnancy and attributed it to increased emotional stress as a pregnant mother is expecting a child and decreased level of vaginal defense mechanisms. Vaginal *Candida* species occurred less frequently in the first and second trimesters of pregnancy due to the fact that women in those trimesters of pregnancy have reduced emotional stress and strong defense mechanism against *Candida* species. *Candida albicans* was the most frequently vaginal *Candida* species causing vaginitis in the three trimesters of pregnancy indicating that it is the most common cause of vaginitis in pregnant women.

5. Conclusions

The prevalence of *Candida* species among vaginitis symptomatic pregnant women studied was high in the women within the age group of 26-30 years and in those women in their third trimester of pregnancy. *Candida albicans* was the most common of the vaginal candida species isolated and it occurred in all age groups and trimesters of pregnancy, therefore routine medical examination, adequate ante-natal services and appropriate treatment of the infected women are inevitable.

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