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PREVALENCE OF VULVOVAGINAL CANDIDIASIS AMONG PREGNANT AND NON- PREGNANT WOMEN ATTENDING MURTALA MUHAMMAD SPECIALIST HOSPITAL, KANO STATE, NIGERIA

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ABSTRACT
Vaginal candidiasis is a common gynaecological problem among women of childbearing age worldwide. The study aimed to determine the prevalence of vulvovaginal candidiasis among pregnant and non-pregnant women attending Murtala Muhammad Specialist Hospital, Kano State, Nigeria. A total of 300 high vaginal swab samples were collected from women attending antenatal and postnatal clinic of Murtala Muhammad Specialist Hospital, Kano State, Nigeria. All samples were inoculated on Sabouraud Dextrose Agar in duplicate for 7 days at 37°C and 25°C, respectively. The positive cultures isolated were examined morphologically, microscopically, and confirmed using the standard biochemical tests with analytical profile index Candida kit. The confirmed mycological isolates were subjected to anti- fungal sensitivity test using agar well diffusion technique with standard antifungal drugs in various concentrations. Of the 300 samples screened, an overall prevalence of 98 (32.7%) was recorded, with 54 (36.0%) from pregnant women and 44 (29.3%) from non-pregnant women. Out of the 98 positive fungal infection, 67 (22.3%) were* C. albicans, *15 (5.0%) were* C. tropicalis, *7 (2.3%) were C. glabrata and 9 (3.0%) were C. krusei. Amphotericin B showed higher activity against most of the isolated* Candida *species, while majority of the isolates exhibited varying resistance to Ketoconazole. The study showed a higher prevalence of vaginal candidiasis among pregnant than non-pregnant women, which may be due to an increase in the production of progesterone and estrogen, misused, and overused of antibiotics.

Keywords: Antifungal activity, Candidiasis, Non-pregnant women, Pregnant women,

INTRODUCTION

Vaginal candidiasis is a common gynaecological problem among women of childbearing age worldwide. Up to 75.0% of sexually active women will have experienced at least one episode of symptomatic vaginal candidiasis in their lifetime and about 40-50% will suffer from a recurrence (Goncalves *et al.*, 2016). Candidiasis is a disease condition coursed by fungi of the genus Candida (Sule *et al.*, 2020). Among the species of Candida causing vaginal candidiasis, *Candida albican* is the predominant species that cause 80 – 90% of vaginal candidiasis, only a minority of cases 10 – 20% is caused by non-albican species, usually caused by *Candida glabrata* (Sobel, 2007). Candida species form part of normal flora in the women's genital tract of about 20 – 50% of the healthy asymptomatic women (Akah *et al.*, 2010).

Factors associated with an increased rate of vaginal colonization by Candida species include pregnancy, use of high estrogen content drugs and oral contraceptives, uncontrolled diabetes mellitus, prolonged use of broad-spectrum antibiotics which kill the good and beneficial bacteria, allowing yeast overgrowth, poor dietary habits, and poor personal hygiene. Many practitioners believe that nylon underwear and tight insulating clothing predispose to vaginal candidiasis by increasing the temperature and moisture of the perineum (Alli *et al.*, 2011).

Vaginal candidiasis was found to have a serious social, pathological and physiological impact on the woman.

Several *Candida* infections appear to originate from an endogenous source, even though exogenous spread of the infection among patients is possible usually during nosocomial infection (Pfaller, *et al.,* 2014; Criseo *et al.,* 2015). *Candida* species such as *C. albicans, C. tropicalis* and *C. glabrata* constitute part of the common mammalian flora and are found mainly on the mucosal surfaces of the oral cavity, gastrointestinal tract (GI), and female genitourinary tract (Sobel, 2007; Vazquez and Sobel, 2011). These species cause endogenous *Candida* infection.The study aimed to determine the prevalence of vulvovaginal candidiasis among pregnant and non-pregnant women attending Murtala Muhammad Specialist Hospital, Kano State, Nigeria.

MATERIALS AND METHODS

Study Area

The study was carried out at Murtala Muhammad Specialist Hospital (MMSH) Kano, The Capital city of Kano State Nigeria, located between latitudes10° 33'N to 11° 15'N and longitudes 34CE to 8° 20CE (NBS, 2018).

Study Population

The study populations were pregnant and non-pregnant women attending antenatal and postnatal clinic of Murtala Muhammad Specialist Hospitals.

Ethical Consideration

Ethical approval was obtained from the ethical committee of the Kano State Ministry of Health before the commencement of the study with reference number (SHREC/2021/2268).

Inclusion and Exclusion Criteria

Patients included for this study are consented women who visited antenatal and postnatal clinic of the selected hospital, irrespective of their trimester, age, parity, and socioeconomic status. Symptomatic patients aged ≥18years, but without vaginal bleeding.

Determination of Sample Size

The sample size for the study was determined using the cochran formula (Araoye 2004) and based on 13.0% prevalence reported by Ali *et al.* (2018)

$$n = \frac{Z^2 Pq}{d^2}$$

Where:

Where: n= number of samples

Z = statistic for level of confidence at 95% = 1.96

P = a 13.0% prevalence was used, from previous study done by Ali *et al.*, (2018)

d = allowable error of 5% (0.05)

q = 1 - P

$$n = \frac{1.96^2(0.13)(1-0.13)}{0.05^2}$$

n = 173.79 + (5% attrition = 8.69)

n = 182.48

n= 182

Therefore, the number of sample was round up to 300, including 150 pregnant and 150 non-pregnant women

Questionnaire Administration

Data of each participant were collected using a structured and interviewer-administered questionnaire. The questionnaire evaluated the subject's bio-data, socio-demographic and risk factors.

Samples Collection

Three hundred (300) high vaginal swab (HVS) samples that comprise of 150 pregnant and 150 non-pregnant women were collected using sterile swab sticks by certified medical personnel. The samples were labelled appropriately and taken to the laboratory immediately for analysis (Cheesbrough, 2010)

Sample Processing

Primary Isolation

All collected HVS samples were cultured on the Petri dish containing Sabouraud Dextrose Agar (SDA) and incubated aerobically at 37^{o}C for 24 hours (Cheesbrough, 2010).

Wet Preparation Microscopy

After culturing the swab stick containing HVS samples were aseptically added sterile normal saline and mixed thoroughly. A drop of the mixture was placed on a clean, grease-free microscope slide, covered with a coverslip, and examined under a light microscope using X10 objective lens to observe for the presence of yeast cells, followed by confirmation with X40 objective lens (Cheesbrough, 2010).

Procedure for fungal identification

All samples that showed evidence of growth on solid SDA were identified and confirmed using colony morphological characters and biochemical tests, which included Gram stating, germ tube test, and analytical profile index (API) Candida identification kit:

Gram Staining

Gram staining of the isolated fungi was carried out to identify the Gram reaction of the isolates as described by Cheesbrough, (2010).

Germ Tube Test

A germ tube test was done to differentiate *Candida albicans* from *Candida* non-albican that produces a short hyphal (filamentous) extension arising laterally from a yeast cell, with no constriction at the point of origin, after incubated for 2 hours at 37^{o}C in serum (Yan *et al*, 2013).

Preparation of McFarland Turbidity Standard

Preparation of 0.5 McFarland standard (turbidity standard) Sulfuric sulfate (1% v/v) standard suspension was used as turbidity standard which was prepared following the procedure explained by Cheesbrough (2010).

Analytical Profile Index (API) *Candida* Identification Kit

All the germ tube negative Candida isolates were further confirmed by the API Candida identification kit multi-test system (Biomeniuex, France). These tests were used according to the manufacturer's protocol for the identification of Candida species. Wells containing the sugars were inoculated with fungal suspension made from fresh fungal culture. The suspension matched with 0.5 McFarland turbidity standards and incubated at 37^{o}C for 24 hours. The result was read after the addition of the reagent by generating the 4-digit number that identifies the API analytical index (API Candida, Biomeriux, France).

Data Analysis

Data Obtained was analyzed using statistical packaged for social sciences (SPSS) Version 25 (2020, IBM Califonia, USA). The prevalence of fungal species was expressed in simple frequency and percentages for the study groups. The Chi-square test was used to assess the association between the categorical variant and socio–demographical factors of the subject. A p-value of ≤0.05 and considered significant at 95% CI.

RESULTS

Out of 300 samples collected that comprised of 150 (50.0%) from pregnant and 150(50.0%) from non-pregnant women, 98 (32.7%) with 54 (36.0%) from pregnant women and 44 (29.3%) from non-pregnant women, respectively (P=0.268) (Table 1).

From the 98 positive fungal isolates found, 67 (22.3%) were *C. albican* and the remaining were non-albican comprising of 15 (5.0%) *C. tropicalis*, 9 (2.3%) *C. krusei*, and 7 (3.0%) *C. glabrata* (Table 2).

Table 3 showed the relationship between the participant's socio-demographical parameters and fungal culture results and a statistically significant difference as all their calculated p-values are greater than 0.050. Among all the observed possible risk factors, participants on antibiotics drugs had the highest number 53(17.7%) of positive fungal growth, itching 68(22.7%) serve as the major experienced sign of infection reported by the study participants with positive culture result followed by 15(5.0%) with discharge and unpleasant odour each. Concerning the gestation period of the pregnant women, the highest incidence of 26(17.3%) was observed in the first trimester followed by 23(15.3%) among those in the second trimester. The study showed a statistically significant difference between p-value >0.050 (Table 4).

Table 1: Prevalence of Candida Infection In Relation to Pregnancy

Pregnancy Status	**No. Tested**	**No. Positive**	**(%) Prevalence**	χ^2	**df**	***P-value***
Pregnant	150	54	18.0	1.228	1	0.268
Non Pregnant	150	44	14.7			
Total	**300**	**98**	**32.7**			

Table 2: Species Specific Prevalence of *Candida* Infections (n = 98)

Fungal Isolates	**No. Tested**	**No. Positive**	**(%) Percentage**
Candida albicans	98	67	22.3
Non-albican			
C. tropicalis	98	15	5.0
C. glabrata	98	7	2.3
C. krusei	98	9	3.0

Table 3: Percentage Prevalence of Fungal Growth in Relationship to Participants Socio-Demographic Parameters

Socio-Demographic Parameters	No. Tested	Result		χ2	df	*P-value*
		Growth	No-Growth			
Age (Years)						
18 – 25	189(63.0)	54(18.0)	135(45.0)	4.284	2	0.116
26 – 33	96(32.0)	37(12.3)	59(19.7)			
34 – 40	15(5.0)	7(2.3)	8(2.7)			
Total	**300(100.0)**	**98(32.7)**	**202(67.3)**			
Address						
Rural	173(57.7)	61(20.3)	112(37.3)	0.987	1	0.321
Urban	127(42.3)	37(12.3)	90(30.0)			
Total	**300(100.0)**	**98(32.7)**	**202(67.3)**			
Educational Level						
Primary	75(25.0)	24(8.0)	51(17.0)	5.458	3	0.141
Secondary	103(34.3)	31(10.3)	72(24.0)			
Tertiary	15(5.0)	9(3.0)	6(2.0)			
Informal	107(35.7)	34(11.3)	73(24.3)			
Total	**300(100.0)**	**98(32.7)**	**202(67.3)**			

Table 4 Percentage Prevalence of Fungal Growth in Relationship to Risk Factors

Possible Risk Factors	No. Tested	Result		χ2	df	P-value
		Growth	No-Growth			
On Antibiotics						
Yes	165(55.00)	53(17.7)	112(37.3)	0.010	1	0.921
No	135(45.0)	45(15.0)	90(30.0)			
Total	**300(100.0)**	**98(32.7)**	**202(67.3)**			
Type of Underwear						
Nylon	201(67.0)	65(21.7)	136(45.3)	0.002	1	0.967
Cotton	99(33.0)	33(11.0)	66(22.0)			
Total	**300(100.0)**	**98(32.7)**	**202(67.3)**			
Gestation (Period)						
First Trimester	84(56.0)	26(17.3)	58(38.7)	2.221	**2**	0.329
Second Trimester	53(35.3)	23(15.3)	30(20.0)			
Third Trimester	13(8.7)	5(3.4)	8(5.3)			
Total	**150(100.0)**	**54(36.0)**	**96(64.0)**			
Symptoms						
Discharge	55(18.3)	15(5.0)	40(13.3)	0.932	2	0.627
Itching	199(66.3)	68(22.7)	131(43.7)			
Odour	46(15.3)	15(5.0)	31(10.3)			
Total	**300(100.0)**	**98(32.7)**	**202(67.3)**			
Duration of Sign (Week)						
<1	170 (57.0)	53 (17.7)	117 (39.0)	0.595	2	0.743
1 – 3	67 (22.3)	22 (7.3)	45 (15.0)			
>3	63 (21.0)	23 (7.7)	40 (13.3)			
Total	**300(100.0)**	**98(32.7)**	**202(67.3)**			

Key: **χ2 =**chi square, df =degree of freedom, P-value=probability value

Table 5 The Susceptibility of *Candida* species to antifungal agent Antifungal agents Susceptibility

Antifungal Agent	*C. albicans* (N=67)			*C. tropicalis* (N=15)			*C. glabrata* (N=7)			*C. krusei* (N=9)		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Amphotericin B	59(88.1)	5(7.5)	3(4.5)	14(93.3)	1(6.6)	0(0.0)	6(85.7)	1(14.2)	0(0.0)	6(66.6)	1(11.1)	2(22.2)
Clotrimazole	51(76.1)	11(16.4)	5(7.5)	12(80.0)	3(20.0)	0(0.0)	6(85.7)	0(0.0)	1(14.2)	9(100.0)	0(0.0)	0(0.0)
Nystatin	47(70.1)	18(26.8)	2(3.0)	14(93.3)	1(6.6)	0(0.0)	4(57.1)	2(28.5)	1(14.2)	8(88.8)	1(11.1)	0(0.0)
Itraconazole	39(58.2)	21(31.3)	7(10.4)	8(53.3)	4(26.6)	3(20.0)	7(100.00)	0(0.0)	0(0.0)	6(66.6)	2(22.2)	1(11.1)
Ketoconazole	22(32.8)	13(19.4)	32(47.7)	0(0.0)	4(26.6)	11(73.3)	0(0.0)	1(14.2)	6(85.7)	1(11.1)	1(11.1)	7(77.7)
Fluconazole	15(22.3)	8(11.9)	44(65.6)	9(60.0)	3(20.0)	2(13.3)	4(57.1)	0(0.0)	3(42.8)	3(33.3)	6(66.7)	0(0.0)

Key:- S: Sensitivity, R: Resistance, I: Intermediate

DISCUSSION

The result of the present study showed the presence of high prevalence rate of candidiasis of 32.7% among the studies subjects with pregnant women 18.0% exhibiting higher prevalence than non-pregnant women 14.7%. The higher prevalence of *Candida* infections in pregnant women compared to non-pregnant women reported in this study agreed with the finding of Mumuney and Abalaka (2019) who reported a higher frequency of 57.5% and 20.0% Vaginal Candidiasis among pregnant women and non-pregnant women, respectively in their study done at General hospital Minna and IBB Specialist Hospital, Minna, Niger State. Similarly, Sampson and George (2019) in Port Harcourt, Rivers State, and Ekelozie *et al.* (2018) in Benin city, Edo State, Nigeria, were also reported the vaginal candidiasis prevalence of 43.0% among pregnant women, 29.0% in non-pregnant women and 58.0% in pregnant women and 40.0% among the non-pregnant women, respectively. This variation between pregnant and non-pregnant women is due to an increase in the production of progesterone and estrogen levels in your pregnant women's body which can throw off the normal balance of yeast in their vagina which causes overgrowth of the *Candida* species (Waikhom *et al*., 2020).

Candida albicans is the most common cause of candidiasis which remains the most frequent specie isolated as recorded in this study. *Candida albicans* accounted for 22.3% of the species isolated in this study. This could be attributed to the fact that *Candida* species are members of the vaginal mycobiota and *Candida albicans* possess the distinctive characteristic feature of dimorphic transition whereby morphological transition from yeast form which is usually found in healthy asymptomatic women can transit to hyphal form, which has consistently been isolated from cases of severe vaginal candidiasis (Adogo *et al*., 2020). The distribution of *C. albicans* in this study agrees with the findings of Khattab (2021), Adego *et al.* (2020) and Omote *et al.* (2018). The relatively high incidence of *C. albicans* in this work could be due to the tolerance of this *Candida* species to acid conditions found in the vagina due to vaginal secretions.

Concerning the age groups of the subjects, the age range of 18 – 25 years had the highest infection rate 18.0% followed by 26 – 33 years 12.3% while the age least was found among aged 34 – 40 years 2.3%. The high prevalence rate among the women of such age group may be due to high sexual activity, the use of contraceptives, and drug abuse among this age group, and also advancement in age, on the other hand, reduces the effect of estrogen hormone in women, which could lead to lower infection rates as women advance in age (Azike *et al.*, 2019). The finding of this study agreed with what was reported by Sule *et al.* (2020) and Aliyu *et al.* (2019).

As other studies indicated, the prevalence of vaginal candidiasis is relatively associated with the address and educational status of the women participants, higher (20.3%) among women from rural areas than those in urban areas (12.3%). Informal education, secondary and primary school, and tertiary school groups gave a prevalence of 11.3%, 10.3%, and 8.0% respectively, while tertiary degree holders had a 3.0% prevalence. These tallies with the findings of Dike-Ndudim *et al.* (2021) and Ezeigbo *et al.* (2015). Inadequate knowledge, poor personal hygiene, limited diagnostic facilities; poor dietary habits may be responsible for the high prevalence of vaginal candidiasis (Mumuney and Abalaka, 2019).

In this study, 17.7% of the participants with vaginal candidiasis are on antibiotics treatment. Although, the study showed a statistically significant difference between the antibiotics used and the presence of vaginal candidiasis among the study groups. This agrees with another study done by Mumuney and Abalaka (2019) and that of Omote *et al.* (2018) that all believed abuse of spectrum antibiotics can serve as the risk factors associated with the incidence of vaginal candidiasis. The study confirmed that vaginal itching among one of the most important signs of vaginal candidiasis. This is in concordance with the result of other studies done by Dike-Ndudim *et al.* (2021), and Ekelozie *et al.* (2018) where all suggested that vaginal itching is among the patient's signs of vaginal candidiasis.

The occurrence of vaginal candidiasis based on trimester indicated that pregnant women in the first trimester of pregnancy, had the highest prevalence of 17.3%, followed by the second trimester with 15.3% and least of 3.3% was from the third trimester of pregnancy. This high occurrence of *Candida* recorded in the first and second trimester may likely be a result of fetal demand for nutrients. This is agreed with an earlier report by Jombo *et al.* (2010). Whereas in contrast with the work of Nelson *et al.*, (2013), who found that pregnant women in their third trimester had the highest prevalence of vagina candidiasis (68.09%) followed by the second trimester (21.28%) and the least in the first trimester (10.63%).

CONCLUSION

The present study showed high prevalence of vaginal candidiasis of 32.7% among the study subjects 54 (18.0%) from pregnant women and 44 (14.7%) from non-pregnant women, respectively. *Candida albical* (22.3%) was found to be the predominant isolates causing vaginal candidiasis among the study groups, followed by *C. tropicalis* (5.0%). Most *Candida* isolates in our study were resistance to ketoconazole and fluconazole. However, amphotericin B, Itaraconazole and Clotrimazole recorded the highest susceptibility pattern. The study also revealed that misused of broad-spectrum antibiotics, can serve as possible risk factor for vaginal candidiasis, so as stage of pregnancy stage.

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