Prevalence of Candidemia among Pregnant Women Attending Antenatal Clinic, Muhammad Abdullahi Wase Teaching Hospital, Kano-Nigeria

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Abstract

As opportunistic pathogens, Candida species initiate infection from any susceptible part of the body. Common diseases caused by these species include oral thrush, oropharyngeal Candidiasis, and vulvovaginal Candidiasis (especially during pregnancy). The pathogens disseminate from particular sources to the bloodstream, causing candidemia. The study aimed to determine the burden of candidemia among pregnant women in the study area by collecting 269 blood samples and analyzing them through Gram staining, germ tube tests, and culture-based techniques to phenotypically identify the different Candida species. The results showed an overall prevalence of 10.4%. Candida albicans was the most isolated species (46.43% prevalence), followed by C. krusei (25.0%), C. glabrata (21.43%), and Candida tropicalis (7.14%). Of the participants recruited, 32.0% were in the first trimester, 29.0% were in the second trimester, and 39.0% were found to be in their third trimester. Infections in the third trimester were higher than in the first and second trimesters (50% and 25%, respectively), and statistically, the trimester is linked to the infection when compared using the chi-square test (p = 0.00001). The prevalence of candidemia, based on age range, showed that those between the age group of 20-29 years had a higher number of positive cases (54.0%), while the age bracket less than 20 (<20) years of age had the least 14.0%). Therefore, It can be concluded that candidemia in pregnancy is a common feature, with a significant proportion found in the third trimester as in the study area. Based on the findings, screening for candidemia in pregnancy is recommended to better manage possible cases and reduce the chances of complications.

Keywords: Antenal clinic, Candida species, Candidemia, Pregnant women

INTRODUCTION

Candidiasis is considered among the important fungal infections associated with humans worldwide, which is caused by different types of *Candida* species with varying degrees of isolation rates, depending on the region and category of hosts involved. Candida is believed to consist of over 350 heterogeneous species, among which relatively few are known to be involved in opportunistic human diseases (Williams *et al.*, 2011).

It is widely believed that vaginal Candidiasis in pregnancy sometimes leads to bloodstream infections, particularly associated with low birth weight and premature infants. Interestingly, nowadays, due to molecular typing techniques, vertical transmission of *Candida* species like *C. glabrata, C. parapsilosis,* and *C. albicans,* among others, were identified (Bliss *et al.*, 2008). A reported study from Malaysia related to the epidemiology of vaginal Candidiasis in pregnancy showed that the majority of cases are associated with bloodstream infection (Chong *et al.*, 2003).

Candida infection is one of the most common forms of fungal disease that involves different parts of the human body, including the urogenital tract of women, especially when candida reaches the bloodstream (Kamath *et al.*, 2013). The *Candida* species are the major cause of vaginitis among women. They are second only to bacteria, especially in pregnancy. The disease is characterized by thick white vaginal discharge, vaginal pruritis, itching, and vulval inflammation (Hainer and Gibson, 2011). Based on clinical evaluation, vaginal Candidiasis leading to candidemia can be classified as either simpler/uncomplicated or more problematic/complicated forms. In this case, uncomplicated vaginal *Candidiasis* is usually caused by *C. albicans,* and it leads to mild to moderate symptoms, while complicated vaginal

Candidiasis is often frequent among immune-compromised subjects and pregnant women and can lead to candidemia or bloodstream infection (Hainer and Gibson, 2011).

During their reproductive age, women normally encounter at least an episode of vaginal Candidiasis in their lifetime (Pakshir and Kimiaghalam, 2007). However, there exist some predisposing factors that increase the risk of developing vaginal Candidiasis. These include the use of contraceptives, diabetes, HIV infection, the use of broad-spectrum antibiotics, and pregnancy in women (Salehi, 2012). Although it is a common problem among women at their reproductive age, the incidence of candidemia tends to be higher in pregnancy because of the expected natural physiological changes that favor the growth of *Candida* in the genitourinary tract (Coutinho, 2009).

Additionally, during pregnancy, the reproductive hormone levels, such as estrogen and progesterone, are usually elevated, suppressing neutrophil's anti-*Candida* activity. This also inhibits the activity of vaginal epithelial cells, which can subsequently lead to candidemia (Coutinho, 2009). Also, in pregnancy, estrogen causes a decrease in immunoglobulins secretion in the vagina, leading to increased vulnerability of women to vaginal Candidiasis (Aslam *et al.*, 2008). Pregnancy also helps in building high glycogen content in the vagina, which serves as a carbon source for *Candida* species to successfully grow (Coutinho, 2009).

Sometimes, in normal pregnancy, candidiasis does not expose the fetus to significant risk, except if disseminated into the bloodstream, causing candidemia. That is when the fetus is at greater risk. However, the fetus can sometimes be affected by vulvovaginal Candidiasis as a result of candidemia if not effectively treated (Oviasogie and Okungbowa, 2009). Another compounding problem noticed is the frequent development of resistance by *Candida* species against most antifungals used for treatment, including cases of candidemia (Al-akeel *et al.*, 2013). Accordingly, the study assessed the prevalence of candidemia level among pregnant women in the study area.

MATERIALS AND METHODS

Study area

The study was conducted in Muhammad Abdullahi Wase Teaching Hospital, Kano, in Nassarawa Local Government, Kano State. The state lies between latitude 11050′ to 12007′ N and longitude 8022′ to 8047′ E, and altitude 472 meters above sea level. The total land area of Kano State is 20,760 sq kilometers with a population of 9,383,682 based on the official 2006 National Population and Housing Census (Ado, 2009).

Sample size determination

This was done using the formula:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where:

n=sample size

Z=confidence interval (90%) =1.64

P=prevalence (50%) = 0.5

d=desired accuracy= 5%=0.05

$$n = \frac{1.64^2 \times 0.5(1-0.5)}{0.05^2} = 269.$$

Ethical approval and informed consent

Ethical approval to conduct the research was obtained from the Ministry of Health Kano State (reference no: SHRFC/2021/2353). In contrast, the participants' verbal and written consents, as the case may be, were also obtained before administering the questionnaires to the enrolled subjects.

Sample collection and analysis

Five (5 mL) blood samples were collected aseptically into labeled vacutainer tubes, and each sample was processed immediately, but when delay was anticipated, the samples were stored in the fridge at 8°C before being used (Nwanze, 2010).

Culture methods

The blood samples were introduced into thioglycolate broth and incubated at 37°C for 72 hours, after which it was subcultured onto Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 24 hours for any possible growth (John, 2012).

Microscopy

Gram's staining technique

The smear was made of the colonies produced by placing a few colonies in a drop of normal

saline on a clean, grease-free slide, emulsified, air-dried, and heat-fixed by quickly passing over the flame three times. The smear was flooded with the primary stain (crystal violet) applied for 1 minute and rinsed with water, then covered with Lugold's iodine for 1 minute, decolorized with acetone for a few seconds, rinsed with water then counterstained with neutral red for 1 minute, rinsed with water then allowed to air dry and examine microscopically using 100x objective lens (Ochei, 2007). A gram-positive large oval budding yeast indicates *Candida* species morphology.

Germ tube test

A small volume of serum (0.5 mL) was dispensed into small test tubes. The colonies from pure culture were picked and gently emulsified in the serum and incubated at 37°C for 3 hours. A preparation drop was transferred onto a clean, grease-free slide, covered with a cover slip, and then examined using a 40x objective (Ochei, 2002). Oval yeast with short cylinder-like protrusions is suggestive of *Candida albicans*.

Inoculation on chromogenic agar for speciation

The isolates obtained from pure culture on SDA were subcultured onto chromo *Candida* agar and incubated at 37°C for 24 hours. After the incubation period, the colonies were differentiated base on the colour produced by the species (John, 2012). The different *Candida* species are identified based on the different colours they produce on chrome agar after incubation. For example, *Candida albican* is green, while *Candida tropicalis* is blue.

Statistical analysis

The data generated in this study was analyzed using the Chi-Square test, calculated using the Statistical Package for Social Sciences (SPSS, version 20.0), with a p-value of $\leq$ 0.05 considered significant.

RESULT

Out of the 269 participants, 28 (10.4%) were found to harbour *Candida* species. With respect to the species isolated, *Candida albicans,* was the species with the highest isolation rate, 46.43%, followed by *C. krusei* 25.0%, *C. glabrata* 21.43%, and *Candida tropicalis* 7.14% as shown in Table 1. Out of the 269 pregnant women recruited, 32.0% were in the first trimester, 29.0% were in the second trimester, and 39.0% were found to be in the third trimester. For trimester-related infection, the higher percentage was found in the third trimester at 50.0%, while the first and second trimesters had 25% each, Table 2. On the candidemia result based on age range, those between the age group of 20-29 years had a higher number of positive cases with 54%.0 while the age bracket less than 20 years (<20) of age had the least number of positive cases, 14.0% as seen in Table 3.

Table 1: Frequency of *Candida* species isolated among pregnant women attending antenatal clinic, Abdullahi Wase Teaching Hospital (N=269)

Species isolated	No. examined (269)	No. Positive (%)
C.albican		13(46.43)
C.krusei		7(25.0)
C.glabrata		6(21.43)
C.tropicalis		2(7.14)
Total	**269**	**28(100)**

Table 2: Prevalence of candidemia based on trimester among pregnant women

Trimester	No. Examined	No. of positive%	No. of negative%	X^2	p-value
				33.00	0.00001
First	86.0	7(25.0)	79(32.8)		
Second	78.0	7(25.0)	71(29.5)		
Third	105	14(50.0)	91(37.7)		
Total	**269**	**28(100.0)**	**241(89.6)**		

Table 3: Occurrence of the disease with respect to age group

Age group	No.Examined	Positive(%)	Negative(%)
<20	19	4(14.0)	15(7.0)
20-29	129	15(54.0)	114(47.0)
30-39	121	9(32.0)	112(46.0)
Total	**269**	**28(100)**	**241(100)**

DISCUSSION

The study involved 269 subjects, of which 28, representing 10.4%, was the overall prevalence recorded. This could be due to the relatively weak immune status of women during pregnancy and the increased risk of opportunistic infection. The implication of this, is more risk to the population and increased public health concern. A contrasting result, a 23.4% prevalence, was reported in 2016 in a similar study (Efunshile *et al.,* 2016). Another finding by Donbraye *et al.,* 2010 also revealed a higher result, 26.0% as reported prevalence rate (Donbraye *et al.,* 2010). In the same vein, research involving 300 pregnant women reported 90 positive cases, representing an overall prevalence of 30% in a particular study (Okonkwo *et al.,* 2010). In 2020 also, a prevalence rate of 36% was reported in pregnant women, which was eventually linked to risk for the fetal well-being of the mother (Adewale *et al.,* 2020). Another report also revealed a prevalence rate of 44.8% in 2019, as reported by the researchers (Nahed *et al.,* 2019).

With respect to the species isolated, *Candida albicans* was the species with the highest rate, 46.43%, followed by *C. krusei* 25.0%, *C. glabrata* 21.43%, and *Candida tropicalis* 7.14%, which implies the contribution of *Candida* species in complication associated with bloodstream infection among pregnant women. A similar finding also revealed that *Candida albicans* was the predominant species isolated, accounting for up to 83.5% of the isolates identified, and the next was *Candida glabrata* with 16% while *Candida famata* had only 0.05% as the least isolated (Masri *et al.,* 2015). Also, in 2016, it was observed that *Candida albicans* had 53.5% as the most isolated species, followed by *Candida glabrata* with *a* 14.1% isolation rate (Efunshile *et al.,* 2016). It was also reported in 2020 that C. albicans was the cause of most infections with 231 isolates, C. galbrata with 43 isolates, C. tropicalis with 22 isolates, and *C. krusei* had only 14 isolates (Adewale *et al.,* 2020). However, in contrast to our findings, research revealed that *C. glabrata* was the most isolated species, 44.4% identified while *C. albicans* had 43.4% as the second identified species (Nahed *et al.,* 2019).

Based on age limits, those between the age group of 20-29 years had a higher number of positive cases, with 54%.0, while the age range less than 20 years (<20) had the least positive cases, 14.0%. The findings of this study concerning the link between prevalence and age (20-29 years had the highest while less than 20 years had the lowest prevalence) a re in contrast to a 2019 finding which showed that the age group of 31-40 with the highest rate of infection 31% while age bracket of 20-25 recorded the least infection rate 12.5% as reported by (Nahed *et al.,* 2019).

CONCLUSION

The obtained results from this study indicated th at *Candida* species are among the causative agents of bloodstream infections in pregnant women, with the highest prevalence being in the third trimester. The predominant cause is *Candida albicans*; the age group of 20-29 was the most vulnerable in the study area.

RECOMMENDATION

Screening for candida infection should be integrated into routine tests done for pregnant women during antenatal visits. Prompt treatment should be initiated to prevent the *Candida* species from entering the bloodstream, leading to candidemia.

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