Original Research Article

Comparative study of Candidiasis in pregnant and non-Pregnant women attending Ibrahim Badamasi Babangida Specialist Hospital and General Hospital, Minna Niger state

ABSTRACT

Candidiasis is a fungal infection due to any type of *Candida* (a type of yeast) [2]. When it affects the mouth, it is commonly called thrush. High vaginal swab (HVS) were collected from 20 pregnant and 20 non-pregnant women in General Hospital, Minna and IBB (Ibrahim Badamosi Babangida) Specialist Hospital, Minna, Niger state and screened for the presence of yeast associated with *candidiasis*. Saboroud dextrose agar (SDA) incorporated with chloramphenicol was used to isolate the *Candida* species in the HVS samples. Identification, Characterization and Biochemical test were used for confirmation of the *Candida* isolates. Thirteen (13) samples tested positive to *candida* infection. The species identified were *Candida albicans* (9), *Candida krusei* (2), *Candida tropicalis* (1), and *Candida pseudotropicalis* (1) for pregnant women in General Hospital. Ten (10) samples taken from pregnant women in Ibrahim Babangida Specialist Hospital showed positive reactions for *Candida* infection. Nine (9) out of twenty (20) samples collected from non-pregnant women in General Hospital were *Candida* infection while three (3) out of twenty (20) samples from non-pregnant women from Ibrahim Babangida tested positive to *Candida* infection. Biochemical analysis shows each *Candida* isolate's ability to utilize different sugars during Sugar Fermentation Test. Germ Tube Test was used to identify and differentiate *Candida albicans* from other species of *Candida*.

Keywords: Pregnant women, High vaginal swab (HVS), *Candida albicans,* Non-pregnant women

INTRODUCTION

Candidiasis is a fungal infection due to any type of *Candida* (a type of yeast) [2]. When it affects the mouth, it is commonly called thrush [2]. Signs and symptoms include white patches on the tongue or other areas of the mouth and throat [3]. Other symptoms may include soreness and problems swallowing.[3] When it affects the vagina, it is commonly called a yeast infection [2]. Signs and symptoms include genital itching, burning, and sometimes a white "cottage cheese-like" discharge from the vagina [8]. Less commonly the penis may be affected, resulting in itchiness [3]. Very rarely, the infection may becoming invasive, spreading to other parts of the body [9]. This may result in fevers along with other symptoms depending on the parts involved [9].

More than 20 types of *Candida* can cause infection, with *Candida albicans* being the most common [2]. Infections of the mouth are most common among children less than one month old, the elderly, and those with weak immune systems. Conditions that result in a weak immune system include HIV/AIDS, the medications used after organ transplantation, diabetes, and the use of corticosteroids. Other risks factors include dentures and antibiotic therapy [4] .Vaginal infections occur more commonly during pregnancy, in those with weak immune systems, and following antibiotic use [10]. Risk factors for invasive candidiasis include being in an intensive care unit, following surgery, low birth weight infants, and those with weak immune systems. [11]

Efforts to prevent infections of the mouth include the use of chlorhexidine mouth wash in those with poor immune function and washing out the mouth following the use of inhaled steroids.[5] Little evidence supports probiotics for either prevention or treatment even among those with frequent vaginal infections.[10][11] For infections of the mouth, treatment with topical clotrimazole or nystatin is usually effective. By mouth or intravenous fluconazole, itraconazole, or amphotericin B may be used if these do not work. [5] A number of topical antifungal medications may be used for vaginal infections including clotrimazole. [8] In those with widespread disease, an echinocandin such as caspofungin or micafungin is used. [9] A number of weeks of intravenous amphotericin B may be used as an alternative [11] in certain groups at very high risk, antifungal medications may be used preventatively [11].

Infections of the mouth occur in about 6% of babies less than a month old. About 20% of those receiving chemotherapy for cancer and 20% of those with AIDS also develop the disease [6]. About three-quarters of women have at least one yeast infection at some time during their lives.[7] Widespread disease is rare except in those who have risk factors. [10] This research aimed to carry out comparative study of candidiasis between pregnant women attending General Hospital and IBB Specialized Hospital, both in Minna, Niger state.

MATERIALS AND METHOD

Material used in this research were microscope, swab stick, slide hydrogen peroxide, normal saline, petri dishes, test-tubes, crystal violet, ethyl alcohol, oil immersion, safarine, SDA (sabouraud dextrose agar).

AREA OF STUDY

The studies areas of this research were General hospital Minna and IBB Specialist Hospital, co-located in Minna, Niger State.

STUDY POPULATION

A total of eighty (80) women were studied: 20 pregnant and 20 non-pregnant from each data source. The ages of the subjects were between 15 and 50 years.

MEDIA PREPARATON

The media used, Saboraud dextrose agar (SDA) was prepared in line with manufacturer's instruction.

MEDIA COMPOSITION

65 grams of SDA suspended in 1litre distilled water

0.5grams of Chloramphenicol powder.

The SDS suspension was sterilized by autoclaving at 121°C for 15 minutes. 110ml of the medium was then dispensed into Petri dishes after cooling.

SAMPLE COLLECTION

High vaginal swab was collected using sterile swab stick. The study sample space consisted of eighty subjects without vaginal disorder were studied. The cervix was opened with sterile unlubricated bivalve. Vaginal spectrum specimen of vaginal discharge was collected from the posterior and lateral furnicis. The sample was then transported immediately to the laboratory and inoculated into freshly-prepared SDA and kept on the incubating hood at 28^0C for 48 hours. Growths having milk to white colour and palm wine smell were picked for further identification and characterization.

IDENTIFICTION AND CHARACTERISATION OF *Candida* ISOLATE

Growths of yeast were seen on the petri dishes after 48 hours of incubation at 28°C on the SDA medium. Colonies were counted using colony counter. Smear preparation was made on a clean slide and gram-stained to use the morphological characteristics of the organism.

GRAM STAINING

The Gram staining was performed as described previously by (Cheesbrough, 2008, Oyeleke and Manga, 2008) .The following reagents were used for the staining: Crystal violet stain, Lugol's iodine, Acetone-alcohol decolorizer and Neutral red. The reagents were prepared according to manufacturer's instructions. Suspected *Listeria monocytogenes* bacteria colonies on any of the agar plates were emulsified in Normal saline on the slide to form a smear. The smear was allowed to air dry completely. The slide, (with the smear uppermost) was fixed by rapidly

passing through flame of a Bunsen burner. The smear was allowed to cool. The fixed smear was covered with crystal violet stain for 30 seconds – 1 minute. The stain was rapidly washed off with clean running tap water. The smear was again covered with Lugol's iodine for another 30 seconds – 1 minute and washed off with clean tap water and was rapidly decolourized (few seconds) with acetone-alcohol. This was washed immediately with clean tap water. The smear was then covered with neutral red stain for 2 minutes and washed off with clean tap water. The stained slide was placed in a draining rack and the smear allowed to air dry. The smear was examined microscopically using oil immersion objective lens (×100).

BIOCHEMICAL TEST (FERMENTATION OF SUGAR)

The biochemical test for yeast isolate is sugar fermentation, this test shows how each organism ferments, or utilizes, different types of sugar (glucose, lactose, maltose, galactose, fructose, and sucrose).

Procedure

Two grams of each sugar were weighed into different beakers. One gram of peptone was added to each along with phenol red indicator powder. 100ml of distilled water was added to each beaker and shaken till it dissolved. 10ml of the sugar solution was dispensed inside test tube that had been labelled accordingly and covered with the cotton wrapped with foil paper and then autoclaved at 121^0C for 15minutes. It was allowed to cool after which small quantity of the pure yeast isolated was inoculated inside each test and incubated in the inoculating hood at the room temperature for 3 days. The result of color change was observed and recorded appropriately.

LACTOSE PHENOL COTTON BLUE STAIN PROCEDURE

A glass slide was cleared with clean cotton wool and a drop of lactose phenol cotton blue was dropped on the slide after which a small portion of the pure isolate was picked and smeared with

the lactose phenol on the slide and covered with clean cover slide mounted. It was viewed under a microscope at 10X magnification. The chlamydospores of the organism were clearly visible.

CONFIRMATION OF *Candida albicans*

This is a confirmatory test for the presence of *candida albicans* in a sample

PROCEDURE

A mammalian serum was obtained. 0.5ml of the serum was added in a clean sterile container. Small colony of the pure isolated *Candida* was emulsified with a sterile wire loop and incubated for 3 hours, after which it was smeared onto a clean slide, covered with a slide and viewed under the microscope using X10 objectives lenses. The spore and hyphae of the organism were visible, establishing a positive result for *Candida albicans* only.

RESULTS

C*andidiasis* in relation to age

The result revealed that of the 20 sample screened in pregnant women in General Hospital, Minna, 13 had *Candida*, with the remaining 7 samples testing negative. This is shown in Table 1. Ten of the 20 samples taken from pregnant women in IBB Specialist Hospital Minna tested positive for Candida, shown in Table 2.

Amongst the non-pregnant women study group, 5 of 20 screened at the General Hospital tested positive, with 15 testing negative the result of which is given in Table 3. The study group of IBB Specialist Hospital tested positive to *Candida* in three cases, with 17 testing negative. This is shown in Table 4. The age range mostly affected in non-pregnant women in both hospitals was 21 – 30 years as shown in all the tables.

The identification and frequency of occurrence of *Candida* isolate identified in this research is shown in Table 5. These are *Candida albicans, Candida krusei, Candida pseudotropicalis* and *Candida tropicalis*. *Candida albicans* had the highest frequency occurrence, closely followed by

Candida krusei. The rest two isolate *Candida pseudotropicalis* and *Candida tropicalis* had the least frequency of occurrence in both hospitals.

Table 7 shows the Biochemical and Morphological Characteristic of *Candida* species isolated from the research work. *Candida albicans* was able to utilize Glucose, Maltose, Lactose, Galactose, Fructose, and Sucrose.

The chlamydospore of *Candida albicans* are row-like round ovals budding yeast that form smooth, creamy, and numerous colonies. Pseudomycelia are also numerous.

Candida krusei has no chlamydospore, but are elongated budding cells with occasional pseudohyphae, forming whitish growth, flat, dry, and often small semi-glossy wrinkled colonies. It is capable of utilizing any of the sugars as source of carbon.

Candida tropicalis possesses no chlamydospore, and is characteristically exhibits flat growth with smooth margin. It utilizes only Fructose, glucose, Maltose, and Sucrose as carbon source.

Candida pseudotropicalis has no chlamydospore, and exhibits moist, creamy, round smooth-walled colonies. It utilizes all the sugars with the exception of maltose.

Table1. Number of HVS positive cases of *Candida f*or pregnant women, General Hospital, Minna Niger State.

Age group (years)	Numbers Of Samples Collected	Numbers Of Positives Samples	Numbers Of Negative Samples
11-20	2	1	1
21-30	10	8	2
31-40	7	4	3
41-50	1	0	1
Total	20	13	7

Table2. Number of HVS positive cases of *Candida* in pregnant women attending IBB Specialist Hospital, Minna, Niger State.

AGE GROUP (YEARS) SAMPLE	NUMBER OF SAMPLE COLLECTED	NUMBER OF POSTIVE SAMPLE	NUMBER OF NEGATIVE
11 - 20	2	0	2
21 - 30	10	7	3
31 - 40	7	3	4
41 - 50	1	0	1
TOTAL	20	10	10

Table 3. HVS positive cases of candida for non-pregnant women attending General Hospital, Minna, Niger state.

AGEGROUP (YEARS) SAMPLES	NUMBERSOF SAMPLES COLLECTED	NUMBERSOF POSTIVE SAMPLES	NUMBERSOF NEGATIVE SAMPLES
11-20	2	0	2
21-30	10	3	7
31-40	7	2	5
41-50	1	0	1
TOTAL	20	5	15

Table 4. HVS positive cases of *Candida* for non-pregnant women attending IBB Specialist Hospital Minna, Niger state.

AGE GROUP (YEARS) SAMPLES	NUMBERS OF SAMPLES COLLECTED	NUMBERS OF POSTIVE SAMPLES	NUMBERS OF NEGATIVE SAMPLES
11-20	2	0	2
21-30	10	2	8
31-40	7	1	6
41-50	1	0	1
TOTAL	20	3	17

Table 5. Frequency of occurrence of *Candida* species percentage in pregnant and non-pregnant women attending General Hospital Minna, Niger- State.

Candida species	Number of isolate in pregnant women	Numbers of isolate in in non-pregnant women	Percentage in pregnant women	Percentage in non-pregnant women
C. albicans	9	2	45	10
C. krusei	2	1	10	5
C. tropicalis	1	1	5	5
C. pseudotropicalis	1	1	5	5
Total	13	5		

Table 6. Frequency of occurrence of *Candida s*pecies percentage in pregnant and non-pregnant women attending IBB Hospital Minna Niger State

Candida species	Number of isolate in pregnant women	Numbers of isolate in non-pregnant women	Percentage in pregnant women	Percentage in non-pregnant women
C. albicans	5	2	25	10
C. krusei	3	1	15	5
C. tropicalis	1	0	5	0
C. pseudotropicalis	1	0	5	0
Total	10	5		

Table 7. Biochemical and Morphological Characteristics of the *Candida* isolate (Sugar Fermentation).

Candida Isolates	Morphological Characteristics	Fermentation Sugars
C. albicans	Chlamydospore: round oval in row, oval budding yeast that forms smooth, creamy, and numerous colonies, Pseudomycelium: numerous	Glucose + Maltose + Lactose + Galactose+ Fructose + Sucrose +

C. krusei	No chlamydospore. Elongated budding cells with occasional pseudohyphae. Whitish growth, flat, dry and often with semi-glossy wrinkled small colonies	Glucose - Maltose - Lactose - Galactose- Fructose - Sucrose -
C. tropicalis	No chlamydospore. Flat growth with smooth margin	Glucose + Maltose + Lactose - Galactose + Fructose + Sucrose -
C. pseudotropicalis	No chlamydospore. Moist and creamy, round, smooth-walled colonies	Glucose + Maltose - Galactose + Lactose +

DISCUSSION

The result showed that 45% and 25% of the pregnant women considered in the study in General Hospital and IBB Specialist Hospital respectively had *candida albicans*, while amongst the non-pregnant sample space, the infection rate was 10% for both study locations. This represents a high prevalence of candida infections in pregnant women relative to non-pregnant women.

The statistics indicating a higher incidence of infection amongst the study subjects in General Hospital compared to IBB Specialist Hospital may be attributed to the higher hygiene awareness among users of the Specialist Hospital; this may be premised on the differentials in the financial disposition of the two classes of subjects.

Hormonal changes contribute to a high rate of *Candidiasis* in pregnant women, and up to 90% of women in their third trimester are mostly involved aside from the extreme discomfort of the symptoms compared to non-pregnant women.

Candida albicans poses a threat to newborns, and neonatal thrushes are traced to contact with the mother vagina during birth in infected pregnant women (Talaro, 1996). There also seems to be a trend for recur during pregnancy as a result of the increased level of estrogens and cortoid, the vaginal defence mechanism against such opportunistic infection of *Candida.* This distribution studies showed that are the vast majority of the sufferers in pregnant and non-pregnant women lie between the ages of 21 to 30 years and 31 to 40years. Pregnancy is uncommon in females younger than 15 years or older than 40 years. Most pregnancy occurs between 20 and 35years of age.

The *Candida* species isolated were *C. albicans, C. tropicalis, C. krusei and C pseudotropicalis.* One or more of these were isolated from each positive case of the infection, meaning that were may be multiple etiology. However, these organisms have been reported in cases of mycosis (Haude *et al*, 1980, Talaro and Talaro 1996). Haude *et. al.* 1980) reported that *C. pseudotropicalis* causes oral or genital thrush but rarely meningitis or encephalitis.

CONCLUSION

The study has revealed different *candida* species, in which *C. albicans* has the most frequency in the positive cases in pregnant and non-pregnant women, and others are *C. krusei, C .tropicalis, C. pseudotropicalis.* This suggests etiology, and also how these species of *Candida* ferment types of sugar, and the shapes when emulsified in mammalian serum, and under microscopic examination.

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