



INCIDENCE of VULVOVAGINAL CANDIDIASIS ASSOCIATED WITH *CANDIDA ALBICANS* AMONG PREGNANT WOMEN ATTENDING GOMBE STATE SPECIALIST HOSPITAL, GOMBE, NIGERIA

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ABSTRACT

Vulvovaginal candidiasis (VVC) is a common cause of vaginitis during pregnancy. It is a source of great physical and psychological discomfort as well as suffering. Samples of endocervical and high vaginal swab were collected from 150 women attending Gombe specialist hospital and were inoculated on Saboraud Dextrose Agar (SDA) incorporated with Chloramphenicol to rid the culture of bacterial contaminants. Gram's staining technique and Germ tube test were employed for the identification, as, Candida albicans is positive for both. The gram positive ones were 132 (88%) and were also subjected to germ tube test. 18 (12%) were found to be gram negative. Of the gram positive ones, 120 (91%) were also positive for germ tube test; confirming the presence of Candida albicans. Antifungal susceptibility testing revealed that Ketoconazole was more effective at concentrations of 20, 50 and 100µg/disc with zones of inhibitions of 18.50, 23.50 and 35.50mm respectively, than Griseofulvin (Fulcin) with only 26.00mm zone of inhibition at 100µg/disc concentration.

Keywords: Vulvovaginal, Endocervical, High vaginal swab, Antifungal susceptibility, *Candida albicans*.

INTRODUCTION

Several species of the yeast genus *Candida* are capable of causing candidiasis. They are members of the normal flora of the skin, mucous membrane and gastrointestinal tract. *Candida* species colonize the mucosal surfaces of all humans during, or soon after birth, and the risk of endogenous infection is ever present. Candidiasis is the most common systemic mycosis (Geo *et al.*, 2001).

Vaginal candidiasis is a specific women's genital tract infection (the vagina) caused by *Candida*, a yeast like fungi. Vaginal candidiasis caused by *Candida albicans* occurs at least once in a life time in 75% of women. The women had been ill once candidiasis risk of re-infection persisted for a long time. *Candida* fungus inhabits the intestine and oral mucosa of most healthy people. It can affect women who are pregnant, those who are of age 30-50 years. Approximately 10% of healthy women candidates were determined to vaginal infection caused by *C. albicans* (Mirela *et al.*, 2010).

Vulvovaginal candidiasis is a vaginal mycosis caused by species of the genus *Candida*. It is one of the most common vaginal infections in women, in the fertile period and also the most frequent and most important fungal disease of vaginal content (Sobel, 1996). Women around the world get diagnosed of vaginal candidiasis. Approximately 40-50% of women have repeated infections. Less than 5% of adult female population receives repeated attack of recurrent vulvovaginal candidiasis. Point-prevalence indicates that *Candida* species may be isolated from the genital

tract of approximately 20% (range 10-15%) of asymptomatic health women who are culture positive for *Candida* species in the vaginal area are asymptomatic carriers. The natural history of asymptomatic colonization is unknown, although limited human studies suggest that vaginal carriage may continue for several months and perhaps, years (Mirela *et al.*, 2010).

In addition, many mental and emotional problems are associated with vaginitis (Sobel *et al.*, 1998). Documented risk factors in of vaginal candidiasis are pregnancy (30-40%), use of high estrogen content, oral contraceptives, antibiotics, chemotherapeutic steroids, attendance at sexually transmitted clinic and age (Odd, 1998). The increased secretion of reproductive hormone during pregnancy favours the formation of infection (Sobel *et al.*, 1998). High level of oestrogen provides an increased amount of glycogen in the vagina. Furthermore, glycogen provides a good source of carbon for *Candida* growth and their germination (Mirela *et al.*, 2010). These hormones accelerate the formation of the yeast pseudohyphae. Vaginal candidiasis is rare in post menopausal women and girls due to hormonal dependence of vaginal candidiasis (Sobel *et al.*, 1998). Numerous studies around the world show that *C. albicans* is responsible for the largest number of asymptomatic episodes of vaginal candidiasis. Percentage of infection that causes candidiasis was high in the past decades and varies from 85-90% (Babic *et al.*, 2001 and Odd, 1988).

Candida species in the vaginal mucosa was found in 35% of healthy women (Goldacre *et al.*, 1979). Numerous studies worldwide show that *C. albicans* are responsible for the greatest number of symptoms associated with the vaginal candidiasis. In the past three decades, report shows that there had been an increased percentage of infections caused by non-albican species. These species are often resistant to conventional therapy (Babic *et al.*, 2004). It was demonstrated that oestrogen increases the affinity for the vaginal epithelial cell adherence of candida and yeast cytosol receptor or system to connect to reproductive hormones. These hormones also increase the formation of yeast blastophores as their morphological form (Howard and Kent, 1991). Incidence of candida vulvovaginitis caused by non-albican species increases during the last decade (Kent, 1991) and the risk factors that favour their infection include the uncontrolled use of antifungal agent, incomplete and prolonged use of antifungal agent in the prevention of candida infection.

There is preponderance of vaginal candidiasis in infective vaginal discharge among which studies include 52.5 and 60% isolation rate respectively of *Candida* species (Sobel *et al.*, 1998). Some risk factors for vaginal candidiasis include pregnancy, poorly controlled diabetes, oral contraceptives and antibiotics, immune suppression, douches perfumed feminine hygiene sprays, topical antimicrobial agents, tight clothing, tight underwear, thyroid disorder and corticosteroids (John, 2000).

Vulvovaginal candidiasis caused by the fungus *C. albicans* is approximately 85% of cases, while other species such as *C. glabrata*, *C. krusei*, *C. tropicalis* and *C. stellioidea* rarely cause vaginitis (Van Dyck *et al.*, 1999). Other complications include pelvic inflammatory disease, menstrual disorders, spontaneous abortion and premature birth. It is now well established that the presence of infective vaginal discharge greatly facilitates transmission and acquisition of HIV between sexual partners (FMOH, 2005, Abebe *et al.*, 2001).

Pregnant women with vulvovaginal candidiasis constituted 40% of the total cases seen (Nwadioha *et al.*, 2010). Some studies have implicated pregnancy as an important risk factor for vaginitis (Sobel *et al.*, 1998,) The relationship between pregnancy and vulvovaginal candidiasis indicates increase in hormonal influences and alteration of the vaginal P^H. Immunosuppressed patients recorded 24% risk (Nwadioha *et al.*, 2010).

Systemic conditions such as diabetes mellitus, HIV/AIDS, organ transplant and any chronic debilitating illness can increase the women chances of developing vulvovaginal candidiasis (John, 2000). Depressed cell mediated immunity provides a favourable condition for growth of candida species such as in HIV/AIDS, whereas dysfunction of neutrophils and monocytes favours candidal growth in diabetes mellitus. Broad spectrum antibiotics users posed a 16% risk of vulvovaginal candidiasis, antibiotics and vaginal douching suppress normal bacterial flora can allow *Candida* organism to

proliferate (Nwadioha *et al.*, 2010). Of interest is that, sulphonamides decrease neutrophils, intracellular killing of candida organism, and tetracycline and aminoglycosides have been shown to decrease neutrophil phagocytosis (John, 2000 Nohmi, 1995), while oestrogen has been found to reduce the ability of vaginal epithelial cells to inhibit the growth of *C. albicans* and also decreases immunoglobulin in vaginal secretions resulting in increased vulnerability of pregnant women to vaginal candidiasis (Fidel *et al.*, 2000).

Additional factors like gestational diabetes, frequent antibiotic therapy (Xu and Sobel, 2004). HIV status (Duer *et al.*, 1990), the use of contraceptives (Sobel *et al.*, 1998), reproductive hormones (Fidel *et al.*, 2000 and Nohmi *et al.*, 1995) also predispose women to acute and chronic vulvovaginal candidiasis. The aim of this research therefore was to study the incidence of vaginal candidiasis among pregnant women attending Gombe specialist hospital, Gombe with a view to screening the pregnant women for the incidence of vaginal candidiasis and finding out a better antifungal agent between Ketoconazole and Griseofulvin to cure the ailment caused by *C. albicans* by in-vitro antifungal susceptibility testing.

MATERIALS AND METHODS

Study Area

The research was carried out at Gombe specialist Hospital Gombe state, Nigeria which has a population of about 2.1 million with about 18,000KM². The temperature averages 30°C with an annual rainfall of 52cm.

Women between 20-50 years of age were clinically assessed. They were chosen because they are among the high risk group and as well, are the most vulnerable because of their immune system (John, 2000).

Sample Collection

Endocervical and high vaginal swabs were collected following aseptic precautions (Van Dyck *et al.*, 1999) from pregnant women attending Specialist Hospital Gombe.

Microscopy

A saline mount of the vaginal discharge specimen was covered with a coverslip on a glass slide and was examined microscopically at x400 (Nwadioha *et al.*, 2010) magnifications for the detection of Macro conidia and microconidia of yeast cells.

Culture

A culture of the yeast isolates (*Candida* species) was obtained on Sabouraud Dextrose Agar (SDA) treated with antibiotic, Chloramphenicol to rid the medium of bacterial contaminants. The method of inoculation was a streaking method as described by Tortora *et al.*, (2007) and Cheesbrough (2000). The swab stick containing the vaginal swab obtained from the Hospital was streaked onto the SDA plates. After inoculation of the clinical specimens, plates were incubated at 37°C for 24 hours and then for two days. Colonies of yeast cells that are opaque white to creamy colour were observed as demonstrated by Cheesbrough (2000).

Plates incubated were read as demonstrated by Cheesbrough (2000) based on the colonial characteristics as; size, shape, colour, edge, consistency and diameter.

Gram’s Staining

Small amounts of the fungal colonies were smeared on glass slides and gram stained for observation under the microscope as described by Cheesbrough (2000).

Confirmatory Germ Tube Test

The procedure described by Cheesbrough (2000), was employed. 500µl (0.5ml) of human serum was pipetted into a small test tube. Using a sterile wire loop the yeast colony was inoculated in the serum and then placed in an incubator at 35⁰C-37⁰C for 2-3 hours. Using a Pasteur pipette, a drop of serum yeast was placed on a glass slide, and covered with a cover slip. The preparation was examined using x10 and x40 objectives with the condenser iris diaphragm closed sufficiently to give good contrast. Sprouting yeast cells that are with tube-like outgrowths observed indicated positive germ tube test.

Standardization of Inoculum

Two to three colonies of confirmed *Candida albicans* isolates were picked and incubated into 10ml of a broth incorporated with Chloramphenicol in a test tube. The test tube was then incubated at 37⁰C overnight at ambient air as demonstrated by Mukhtar and Okafor (2002). The overnight broth culture was further diluted with sterile normal saline to a turbidity that matched 0.5McFarland standard (Mackie and McCartney, 1991,).

Preparation of Sensitivity Discs and Bioassay Procedure

Sensitivity discs of 6mm in diameter were punched out of Whatman’s No. 1 filter paper and 100 discs were

put in Bijou bottles, sterilized and kept for further use. 1mg (1,000µg)/ml, 2mg (2,000µg)/ml, 5mg (5,000µg)/ml and 10mg (10,000µg)/ml of the antifungal agents(Ketoconazole and Griseofulvin were prepared using) sterile deionized distilled water as the diluent. These were dispensed in the Bijou bottles containing the filter paper discs and kept at 4⁰C in the refrigerator for further analysis.

The isolates positive for *C. albicans* were subcultured onto the prepared SDA and the plates streaked to a thin smear. The antifungal antibiotic discs were aseptically placed onto the inoculated culture media using sterile forceps and incubated at 37⁰C for 24 hours. Finally, the readings for zones of inhibition were taken and recorded.

RESULTS

Table 1 summarizes the grams staining reaction of 150 samples obtained from 150 pregnant women which indicated that 132(88%) of the samples tested positive and 18(12%) proved negative. The positive result gave a clue for the presence of *C. albicans* which is gram positive and this called for the confirmatory germ tube test. Table 2 shows the result of germ tube test of the 132 gram positive fungal isolates out of which 120 were positive for germ tube test, equivalent to 91% and 12 samples were negative for germ tube which is also equivalent to 9%. This corresponds with the findings of Nwadioha *et al.*, 2010 who reported that out of 420 *Candida* isolated, 354(84%) were *C. albicans*. This result was also in line with the other study of Van Dyck *et al.*, 1999 who reported that vulvovaginal candidiasis is created by the fungi *Candida albicans* approximately by 85% of cases with *C. glabrata* responsible for the remaining 15%.

Table 1: Gram reaction of the fungal isolates

Gram Reaction	Number of samples observed	Percentage occurrence
Positive	132	88%
Negative	18	12%
Total	150	100%

Table 2: Incidence of *Candida albicans* using confirmatory germ tube test

Germ tube test	Number of samples observed	Percentage occurrence
Positive	120	91%
Negative	12	9%
Total	132	100%

Table 3: Sensitivity of *C. albicans* to the two antifungal agents

Antifungal agent	Concentration (µg/disc)/Zone of inhibition(mm)			
	10	20	50	100
Ketoconazole	0.00	18.50	23.50	35.50
Griseofulvin	0.00	0.00	0.00	26.00

DISCUSSION

Table 1 summarizes the Gram’s staining reaction of 150 fungal isolates obtained from specialist hospital which indicated 132 of them as Gram positive (88%) and 18 as Gram negative (12%). The result gave a clue of the presence of *C. albicans* which is gram

positive and this further called for the *germ* tube test for confirmation.

Table 2 shows the result of the germ tube test of the 132 gram positive fungal isolates out of which 120 were positive for germ tube test, which account for to 91%. 12 samples were germ tube negative, equivalent to 9%.

This corresponds with the work of Nwadioha *et al.*, 2010 who reported that out of 420 candida isolated, 354(84%) were *C. albicans*. It is also in line with the study of Van Dyck *et al.*, 1999 who reported that vulvovaginal candidiasis is created by the fungus *C. albicans* approximately by 85%, with *C. glabrata* responsible for the remaining 15%.

In table 3, at 10µg/disc concentration, ketoconazole showed no activity. At the concentrations of concentrations of 20, 50 and 100µg/disc however, the zones of inhibition recorded were 18.50mm, 23.50mm and 35.50mm respectively. This is owing to the fact that ketoconazole is useful in the treatment of both vagina I and mucocutaneous candidiasis due to its good solubility and is systemically easily absorbable.

However, Griseofulvin showed no activity at all the concentrations except at 100µg/disc in which 26.00mm was recorded as the zone of inhibition (Table 3). This must not be unconnected with the fact that Griseofulvin is poorly absorbed and concentrated in the stratum corneum when administered and is only clinically useful in the treatment of dermatophytes infections of the skin, hair and nails and not for the genitourinary tract fungal infections (Geo *et al.*, 2001).

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CONCLUSION

With regards to the result obtained, the incidence of candidiasis was found to be high (91%) among the pregnant women sampled and that ketoconazole was better in the treatment of infection caused by the isolated fungal pathogens. The result reveals that *Candida albicans* was prevalent among the attending pregnant women that patronize Gombe Specialist hospital.

Recommendation

Early diagnosis and prompt treatment of the vaginal candidiasis especially among the risk group in order to avert the complication. Ketoconazole in addition to other tested antifungal agents could be used for the treatment of vaginal candidiasis.

The use of lubricants (Petroleum jelly, olive oil, etc) during sexual intercourse should be avoided as it increases the risk of contracting the disease.

Avoid washing the vaginal area with soap; use water only. Also avoid the use of tight underwear (John, 2000).

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