BJMHR
British Journal of Medical and Health Research
Journal home page: www.bjmhr.com

Association of Vulvovaginal Candidiasis with ABO Blood Group Among Women in a Tertiary Care Hospital, South-Eastern Nigeria

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ABSTRACT

Vulvovaginal candidiasis (VVC) continues to be a common problem worldwide affecting all strata of women in the society despite therapeutic advances. Candida is the most common microorganism responsible for vulvovaginal candidiasis in adolescent women. Owing to the scare, yet inconsistent reports about the possible association between the ABO blood group and vulvovaginal candidiasis, this study was done. A total of 100 subjects aged between 18 and 50 years attending the gynaecology clinic of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi were used for this study. Ethical approval was obtained from the ethical committee of NAUTH. Following analysis and speciation using CHROMagar candida™, a total of 42 Candida species were isolated giving a 42% prevalence for vulvovaginal candidiasis by culture. Three species of Candida were found to be dominant in the vagina of the women studied namely *Candida albicans*, *Candida krusei* and *Candida tropicalis* comprising 61.9%, 28.5% and 9.5% respectively. Results have revealed that there is no association between the ABO blood group and vulvovaginal candidiasis ($p>0.05$)

Keywords: Vulvovaginal, candidiasis, douching, *Candida albicans, Candida krusei, Candida tropicalis.*

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Received 2 July 2016, Accepted 11 July 2016

Please cite this article as: John D *et al*., Association of Vulvovaginal Candidiasis with ABO Blood Group Among Women in a Tertiary Care Hospital, South-Eastern Nigeria. British Journal of Medical and Health Research 2016.

INTRODUCTION

Vulvovaginal candidiasis (VVC) is defined as an episode of acute onset of vaginal soreness, irritation, vulvar burning, itching, dyspareunia, vaginal pH between 4 and 4.5, positive saline or 10% KOH wet-mount microscopic examinations, and a positive yeast culture,[1]. Candida is the most common microorganism responsible for vulvovaginal candidiasis in adolescent women. A survey conducted all over the world indicates that 75% of women will have at least one episode of vaginal candidiasis during their life time, especially during their child-bearing age and, 40-50% of women may experience a recurrence,[2].

Colonization of the vagina requires yeast adherence to vaginal epithelial cells after gaining access through the anal perianal area. *Candida albicans* adheres in significantly higher numbers to such cells than do non-albicans species,[3]. Virulence is enhanced by proteolytic enzymes, toxins, and phospholipase elaborated by yeast,[4].

Overall, the incidence of Candida infections has dramatically increased in recent years as a result of a large increase in HIV/AIDS cases, an ever-expanding population with immunosuppression due to mucosal or cutaneous barrier disruption, defects in the number and function of neutrophils or in cell-mediated immunity, metabolic dysfunction, organ transplantation and extremes of age (<1 year and > 70 years),[5].

In Nigeria it has been documented that *Candida albicans* is responsible for a large majority of vulvovaginal candidiasis (38.4%),[6] while the prevalence of vulvovaginal candidiasis ranges from 30% in pregnant women,[7] to 61.8%,[8].

Nowadays, the interrelation between ABO blood groups and susceptibility to infections has been excessively studied, but the results are not consistent,[9]. Varying reports show for instance that women with blood groups B and AB are more susceptible to urinary tract infections,[10] blood group B is significantly associated with disseminated fungal infections,[11] and that blood group O is a risk factor for *Candida albicans* infections or its oral carriage,[12]

The reason for these discrepancies might be the difference in mechanisms of adherence of these organisms,[13]. Studies have shown that adhesion of *Candida albicans* is mediated by specific interactions with several sugars such as D-mannose, N-acetyl D glucosamine and L-fructose, some of which are the structural dominant sugars of ABO blood group,[14,15].

Despite these scarce, yet inconsistent reports, we are unaware to the best of our knowledge if any association exists between vulvovaginal candidiasis and the ABO blood group; this is perhaps the first report.

MATERIALS AND METHOD

Ethical Approval

Ethical approval was given by the Ethical Committee of the Nnamdi Azikiwe University Teaching Hospital before the commencement of this study.

Subjects

The subjects comprised of 100 women aged between 18 and 50 years and attending the gynaecology clinic of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi. Forty (40) of them were asymptomatic, apparently healthy women of comparable age that served as controls. They gave no history of any major underlying illness neither had they taken antibiotics in the recent past (2-3 weeks before sample collection). The remaining 60 served as the study subjects. Data about all the subjects were collected using questionnaires after obtaining consent, which included information about their age, educational level, occupation, number of sex partners, marital status, parity, prior use of antibiotics, income per month, age at first sex, human immunodeficiency virus (HIV) status and whether or not they douch their vaginas.

Sample Collection and Laboratory Analysis

From each subject, two high vaginal swabs were collected by the attending physician using sterile cotton wool swabs. Also, 0.5ml of venous blood was obtained using sterile needle and syringe into anticoagulated ethylene diamine tetraacetic acid bottle (EDTA). The swab sticks were immediately taken to the laboratory for examination (culture and microscopy). The modified protocol described by,16 was used for the analysis of the samples.

Culture on Mycologic media

One each of the two respective high vaginal swab was used to inoculate on Sabouraud Dextrose agar plate by making a pool at one edge. Using a sterile wire loop, each inoculum pool made on Sabouraud dextrose agar was streaked progressively to obtain discrete colonies while flaming intermittently to avoid cross contamination. The plates were then incubated for growth at room temperature for 48 hours.

Gram Staining

A smear of the swab was made on a clean grease-free slide and heat-fixed. This was placed on a staining rack and flooded with Crystal violet stain for 2 minutes. The stain was washed off under running water and mordanting using Lugols iodine was done for a minute. Upon washing, the smear was decoulourised using Acetone rapidly and now counterstained using the secondary dye (Neutral red) and left for 2 minutes. After washing, the smear was allowed to air dry and observed microscopically at x100 objective.

Wet Preparation

One of each of the two high vaginal swabs taken was used for wet preparation using 10% Potassium Hydroxide (KOH) on a clean grease-free slide and viewed with the ×40 objective to observe for the presence of budding yeast cells, yeast hyphae as well as blastoconidia.

Germ Tube test

The germ tube test was performed according to the method described by, [17]

Chromogenic speciation of Candida Isolates on CHROMagar Candida

Using already prepared CHROMagar Candida plates, the Candida isolates were streaked carefully and incubated for 48 hours before they were read according to the manufacturer's instruction. CHROMagar Candida is a differential medium for the isolation and presumptive identification of clinically important yeasts within 24 and 48 hours on the basis of strongly contrasting colony colours. As such, the light to medium green colonies were identified as *Candida albicans,* steel blue colonies accompanied by purple pigmentation diffused into surrounding agar as *Candida tropicalis* and *Candida krusei* by growth as large, fuzzy, rose coloured colonies with white edges.

Determination of ABO Blood Group and Rhesus (rh) typing

The ABO blood groups for the subjects were determined by the conventional haemagglutination method test using anti A, anti B and anti D sera (Biotec Laboratories Ltd, Great Britain). On a clean grease-free tile, a drop of whole blood was placed each corresponding to a particular antiserum. Upon addition of the antisera (monoclonal antibodies), each was mixed, rocked carefully and observed for the presence of agglutination.

Statistical Analysis

Data obtained from this study were analyzed using percentage prevalence. Statistical package for social sciences (SPSS version 16 using Chi-square and Pearson's correlation) were used to ascertain the relationship between vulvovaginal candidiasis and ABO blood group. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The predominant age group for both the study subjects and control was 21-30 years, comprising about 72% of the total samples used for this study as shown in Table 1

Table 1: Distribution of Subjects and Controls by Age Group

Age Group	Subjects (%)	Controls (%)	Total	Candida species isolated
≤20	6 (10)	6 (15)	12	10
21-30	44 (73)	28 (70)	72	28
31-40	8 (13)	4 (10)	12	4
41-50	2 (3)	2 (5)	4	0
Total	**60**	**40**	**100**	**42**

There were six (6) blood groups as determined by the use of monoclonal antibodies, with O Rhesus 'D' positive being the predominant blood group in both the study and control groups (60%) followed by A Rhesus 'D' positive (22%)

Table 2: Blood Group Distribution and Candida Isolates in Subjects and Controls

Blood Group	Subjects Examined	Subjects Positive	Controls Examined	Controls Positive	Total Isolates positive
A^+	20	14	2	2	
A^-	0	0	2	0	
B^+	2	2	4	0	
B^-	4	0	2	0	
O^+	32	16	28	8	
O^-	2	0	2	0	
	60	**32**	**40**	**10**	**42**

Table 3 shows that three (3) species of Candida namely *Candida albicans*, *Candida krusei* and *Candida tropicalis* were isolated. With respect to the ABO blood group system, O Rhesus 'D' positive had the highest number of Candida species associated with it; while A, B and O Rhesus 'D' negative had no species associated with them. Overall, there was no association between the ABO blood group system and vulvovaginal candidiasis (X^2_{cal}=3.21 < X^2_{tab}=18.307 at 95% confidence interval).

Table 3: Comparison of Candida species in Subject and Control Groups

Blood Group	Subjects with *Candida albicans*	Subjects with *Candida krusei*	Subjects with *Candida tropicalis*	Total	p-value
A^+	4	6	4	14	
A^-	0	0	0	0	
B^+	2	0	0	2	
B^-	0	0	0	0	
O^+	10	4	2	16	0.572
O^-	0	0	0	0	
				32	
	Controls with *Candida albicans*	**Controls with *Candida krusei***	**Controls with *Candida tropicalis***	**Total**	**p-value**
A^+	2	0	0	2	
A^-	0	0	0	0	
B^+	0	0	0	0	
B^-	0	0	0	0	
O^+	8	0	0	8	
O^-	0	0	0	0	
				10	0.102

X^2(df10) =18.3027 (p>0.05)

Inspite of a number of technological advances put in place, vulvovaginal candidiasis remains a common problem worldwide, affecting all strata of society,[4].

A total of forty-two (42) species of Candida were isolated from both the study and control groups, giving an overall prevalence of 42% which is lower than an earlier report of 61.8%,8, but higher than that reported by,[7]. The age group 21-30 years had the highest participation in this study of about 72% and coincidentally the highest number of Candida species was isolated from the group. This is because the age group 21-30 years is the most productive period of a woman and coincides with very high sexual activity in women. The finding of this study agrees with other reports,[18,19] who attributed that to behavioural sexual relations in this age group. In this age group (with no sentiments to sex), there is a seemingly higher tendency for women to have multiple sex partners, oral sex and engage in poor hygienic practices. These are perhaps other reasons why there is a higher prevalence of vulvovaginal candidiasis in this group.

The age group 41-50 had the lowest participation in this study (4%), while no Candida species was isolated from this age group. This agrees with the finding of,[4] that the incidence of vulvovaginal candidiasis peaks in the third decade of life and decline in women older than forty (40) years of age until the permissive effect of oestrogen replacement therapy becomes apparent. Raid and his colleagues,18 also documented similar results in his recent report while comparing vulvovagianal candidiasis in pregnant and non-pregnant women in Saudi Arabia.

Of the six blood groups to which both the study and control groups belonged, only the Rhesus positive subjects gave Candida isolates as there seemed to be a higher relative percentage prevalence of Candida isolates with O^+ blood group. This has been demonstrated by other researchers who did related studies,[9,16]. However, the Rhesus negative blood groups did not show any such semblance even though the exact reason for this is unknown. It is thought that D-mannose, N-acetyl D glucosamine and L-fructose, some of which are the structural dominant sugars of ABO blood group,[14,15] are possessed by the Rhesus positive groups which Candida species are known to have affinity for. This may be the reason why only the Rhesus positive subjects and controls had Candida isolated from them.

Three (3) species of Candida were found in the vaginas of women in Nnewi, Nigeria with vulvovaginal candidiasis namely; *Candida albicans*, *Candida krusei* and *Candida tropicalis* with *Candida albicans* being predominant as reported by earlier studies,[5,20] representing 61.9%, 28.5% and 9.5% respectively. This is very important because in many clinical settings in Nnewi Nigeria, laboratory reports have *Candida albicans* as the only species isolated in women with vulvovaginal candidiasis meanwhile others exist. This may explain the reason

they are poorly managed with antimycotic regimen. The higher prevalence of *Candida albicans* than the other non-albicans species may be related to the presence of more secreted aspartyl proteinases 5 and 9 in *Candida albicans* (SAP 5 and SAP 9), which are known virulence factors in candida species,[21].

This study reports that there is no association (X^2(df10)=18.307, p>0.05 at 95% confidence interval) between ABO blood group and vulvovaginal candidiasis.

Literature is scarce on this subject as this seems to be the first attempt to find a possible link between ABO blood group and vulvovaginal candidiasis in this locality. As such it is difficult to compare the results of this study exclusively with others. However,[16] used the secretor status of individuals (the ability to produce blood group antigens in body fluids such as saliva, tears, sweat, semen and serum) to study the disposition to vulvovaginal candidiasis. That study showed that blood group B (Rhesus factor not indicated) was a risk for vaginal candidiasis (using secretor status), but could not conclusively show a relationship between the ABO blood group and vulvovaginal candidiasis. Also,9 postulated that blood group O (Rhesus factor not indicated) may be a predisposing factor for denture related candidiasis. Not minding these findings, this study made some interesting inputs yet with certain limitations. For instance, the attendant beliefs still prevalent in many African cultures, Nigeria inclusive was brought to the fore during the course of this study. One of such was the belief in using high vaginal swab samples for rituals and this significantly had effect on getting consent from the study population early enough. Also, the methods used to identify the species of Candida were not discriminatory enough owing to financial constrains as only the PCR can give discriminatory results.

CONCLUSION

Conclusively, this study suggests that there is no association between the ABO blood group and vulvovaginal candidiasis. It also reports that three species of Candida are predominant in the vagina of women with vulvovaginal candidiasis in our locality and that *Candida albicans* was found cohabiting with either *Candida tropicalis* or *Candida krusei*, but not *Candida tropicalis* with *Candida krusei*.

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