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The Prevalence of Symptomatic Vulvo-vaginal Candidiasis and *Trichomonas vaginalis* Infection and Associated Risk Factors among Women in the Niger Delta Region of Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author KP designed the study, wrote the protocol and wrote the first draft of the manuscript. Author IJ performed the statistical analysis and managed the analyses of the study. Authors EL and EN managed the literature searches and processed the specimen. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aims to determine the prevalence of symptomatic vulvo-vaginal candidiasis and trichomoniasis and risk factors among women presenting at the outpatient departments of two tertiary health institutions in the Niger Delta region of Nigeria. **Study Design:** A prospective cross-sectional study.

Place and Duration of Study: The study was carried out at the Niger Delta University Teaching

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Hospital (NDUTH), Okolobiri and the Federal Medical Centre (FMC), Yenagoa, both in Bayelsa State, Nigeria between February 2012 and May 2013.

Methodology: 1240 patients presenting with vaginal symptoms: discharge, itching, soreness or burning sensation were consecutively recruited for the study. High vaginal and endocervical swabs were taken from the women and examined microscopically for motile trichomonads and yeasts, and cultured on appropriate media. Gram staining and Germ tube test were used to identify the organisms. A structured questionnaire was used to obtain patient information relevant to the study. IBM SPSS 21 for Windows® was used for statistical analysis. Variables were compared using the Student's t-test and Fisher's Exact test. P values less than 0.05 were considered significant.

Results: The prevalence of VVC was 25.7% (319 subjects), and only *Candida albicans* genus was isolated from the samples. Bacterial culture was positive in 34.3% of the subjects and *Trichomonas vaginalis* identified in 6.5% of the samples. Prevalence of co-infection with *C. albicans* and *T. vaginalis* was 2.6%. Most of the subjects with VVC and *T. vaginalis* infection were in the 21 years to 30 years age group. Diabetes mellitus (p<0.01), being a student (p<0.01) and being single (p<0.01) were significantly associated with *C. albicans* infection and *T. vaginalis* infection. Pregnancy was significantly associated with *C. albicans* infection (p=0.04). The use of low-oestrogen oral contraceptives had no significant association with *C. albicans* or *T. vaginalis* infection.

Conclusion: Symptomatic VVC and *T. vaginalis* infection were found to be common in the Niger Delta region of Nigeria. Risk factors include diabetes mellitus, pregnancy, being single and being a student. Clearly defined clinical guidelines for symptomatic vulvovaginitis management are needed.

Keywords: Vulvo-vaginal candidiasis; trichomoniasis; risk factors; prevalence; symptoms.

1. INTRODUCTION

Infection of the vulva and vagina (vulvovaginitis) is relatively common and can be distressing to patients as they experience discomfort, itching, soreness, a burning sensation and often vaginal discharge. These infections could be caused by bacterial, fungal or protozoal agents.

Candida albicans and *Trichomonas vaginalis* are important causes of vulvovaginitis. *Candida spp.* are usually part of the normal flora of mucocutaneous membranes, of these species, *Candida albicans* is the commonest infectious agent. Infections by *C. glabrata, C. krusei* and *C. tropicalis* have been also described. Most women asymptomatically harbour *Candida* in the vagina [1,2].

Vulvovaginal candidiasis (VVC) has been defined as vulvovaginal inflammation in the presence of candida species and in the absence of other infectious aetiology [3]. VVC is the second most common cause of vaginal infections in the USA after bacterial vaginosis [4,5]. VVC affects mostly healthy women, and *C. albicans* can be isolated from the vaginal tracts of 20% to 30% of healthy asymptomatic non-pregnant women [3]. VVC is believed to occur when the balance between colonization and the host is temporarily disturbed [3]. It is estimated that 75% of all women will develop a yeast infection during their lifetime with 90% caused by *Candida albicans* [6]. Also, about 5% of women with vulvovaginal candidiasis will develop recurrent vulvo-vaginal candidiasis or RVVC (four or more episodes of VVC in the previous year) [7].

Risk factors often associated with VVC include pregnancy [8], uncontrolled diabetes mellitus [9], high-oestrogen oral contraceptives [10,11], genetic predisposition [10,12] and unsafe sexual practices [1,2,11,13,14].

Trichomonas vaginalis which causes trichomoniasis, emerging is an serious reproductive tract pathogen [15]. In 1999, the WHO estimated a prevalence of 174 million new infections per year worldwide [16]. Trichomonas vaginalis is a parasitic protozoan spread mostly sexually, although non-sexual transmission of T. vaginalis has been observed in adolescent girls [17]. T. vaginalis infection is the most prevalent non-viral sexually transmitted infection globally [18]. It mostly affects reproductive-age women, and it is estimated that 85% of Τ. vaginalis infections in women are asymptomatic [19].

Infections with *T. vaginalis* are important because they are associated with reproductive tract problems. *T. vaginalis* infection has been associated with pelvic inflammatory disease [20, 21] and infertility [22]. It can lead to increased

risk of transmission of HIV [23,24], and causes adverse outcomes of pregnancy such as ectopic pregnancy, premature labour, low birth-weight babies [21,25], and also increased risk of cervical cancer [26]. Despite these associated serious consequences, *T. vaginalis* infection is not given the attention it deserves [18].

There is paucity of local epidemiological data on VVC and trichomoniasis due to factors which include inaccurate diagnosis and the use of nonrepresentative study populations [27]. VVC and trichomoniasis in Nigeria are not reportable, and little has been done to verify if the associated risk factors observed in other parts of the world are applicable here. This study, therefore sought to determine the prevalence of both VVC and trichomoniasis and possible risk factors among women with vaginal symptoms presenting at two tertiary health institutions in the Niger Delta region of Nigeria.

2. MATERIALS AND METHODS

This was a prospective cross-sectional study carried out between February 2012 and May 2013 at the outpatient departments of Niger Delta University Teaching Hospital (NDUTH), Okolobiri and the Federal Medical Centre (FMC), Yenagoa, both in Bayelsa State, Nigeria. They are both tertiary health institutions catering for the needs of residents of the state and neighboring states (Rivers and Delta States). A total of 1240 patients presenting with vaginal symptoms: discharge, itching, soreness or burning sensation were consecutively recruited for the study. Patients who had received any form of treatment were excluded. HIV positive women were also excluded from the study.

Ethical approval was obtained from the ethical review board of the Niger Delta University Teaching Hospital. Informed consent was obtained from all the subjects. A structured questionnaire was used to obtain information relevant to the study from the subjects.

Sterile swab-sticks were used to obtain high vaginal (HVS) and endocervical (ECS) specimens by Medical Officers at the Out-patient clinic from the subjects using aseptic techniques and with the aid of a Cusco vaginal speculum. Two specimens were taken per patient, one HVS and one ECS. Specimens were immediately sent to the Microbiology Department for processing.

For each specimen, using a grease-free slide, a saline wet-mount preparation was done and examined microscopically for trichomonads, yeast cells and epithelial cells. A drop of 10% potassium hydroxide (KOH) was then added to each slide and then examined for yeasts/hyphae. In order to discriminate between the various causes of vulvovaginitis, the specimens were inoculated on different media: Blood agar, MacConkey agar, cystine lactose electrolyte deficient (CLED) agar and Sabouraud Dextrose Agar (which is selective for the growth of Candida species). The plates were incubated aerobically at 37℃ for 24 to 48 hours. Gram staining was also done to identify the colonies on the culture media. Fungi appear as large Gram positive ovoid cocci. Germ tube test, hyphae and pseudohyphae growth were used to identify candida species. Women with positive tests were counselled and treated appropriately.

Statistical analysis was conducted using IBM Statistical Package for Social Sciences (SPSS) 21 for Windows[®]. Prevalence of *C. albicans* and *T. vaginalis* for different groups were determined and the proportions were compared using the Student's t-test and Fisher's Exact test. P values less than 0.05 were considered significant.

3. RESULTS

A total of 1240 samples were obtained from patients attending the NDUTH and the FMC (931 subjects and 309 subjects respectively). Their ages ranged from 15 years to 57 years, with mean age of 29.6 years±3.75. Over half of the subjects were in the 21 years to 30 years age group (53.3%) (Table1). Married women accounted for 54.5% of the subjects, with 26.1% of them being homemakers. A quarter of the patients were students. Pregnant women made up 21.5% of the cases, 13.5% were diabetic and only 6.4% had been on oral contraception in the past 6 months. Out of the 1240 patients, 25.7% (319) had positive samples for Candida albicans. Positive bacterial cultures were obtained in 425 samples (34.3%), while Trichomonas vaginalis was identified in the samples of 80 samples (6.5%). Only 2.6% patients (32 cases) were coinfected with T. vaginalis and C. albicans. In 380 (30.6%) subjects, no micro-organisms were visualised or isolated.

To ascertain if the occurrence of *C. albicans* and *T. vaginalis* were age dependent and for computational ease, the subjects were stratified into age groups spanning ten years each. The

prevalence of both *C. albicans* and *T. vaginalis* peaked in the third decade of life and then declined steeply after the fourth decade (Table 1.). There were no statistically significant associations between age and infection with either *C. albicans* or *T. vaginalis*.

Women with diabetes mellitus and pregnant women were more likely to develop vulvo-vaginal candidiasis than the general population. Of the pregnant women, 34.5% had specimens positive for C. albicans. This was significantly higher than in the general population (p = 0.04), while 40.1% of those diabetic had positive samples for C. albicans. This was also statistically significant at p < 0.01 (Table 2.). There was no statistical difference between the use of high-oestrogen oral contraceptives and infection with C. albicans. Majority of the subjects were unwilling to divulge information about their sexual practices or number of sex partners, despite assurances of confidentiality. So, sexual practices could not be explored as a possible risk factor.

Prevalence of *T. vaginalis* was higher among the diabetic subjects (11.37%), than the other groups. This was statistically significant at p = 0.04 (Table 2). However, there was no significant relationship between *C. albicans* infection and pregnancy or use of oral contraception. Diabetes mellitus was also

significantly associated with co-infection of *C. albicans* and *T. vaginalis* p<0.01.

Although students made up just 25.5% of the subjects, 56.5% of them had positive samples for C. albicans. This comprised 55.7% of all C. albicans positive tests and was statistically significant p < 0.01 (Table 3). Being a business woman was also statistically associated with C. albicans infection p=0.03. There was no significant relationship between being a homemaker or a civil servant and C. albicans infection. Trichomonas vaginalis infection was more common among students, who made up 47.5% of those positive for *T. vaginalis*. This was statistically significant at p < 0.01(Table 3). There was however no significant association between other occupation and T. vaginalis infection. Being a student was also significantly associated with co-infection of C. albicans and T. vaginalis p<0.01. None of the subjects was a commercial sex worker.

Single women accounted for 36.9% of the subjects, but 45.4% had samples positive for *C. albicans.* This was statistically significant at p < 0.01 (Table 4). The prevalence of *T. vaginalis* infection was higher among the single subjects, who constituted 76.25% of those infected. Being single was significantly associated with *T. vaginalis* infection (p<0.01) and co-infection with *C. albicans* and *T. vaginalis* (p=0.03).

| Age group | No. of subjects | C. albicans +ve | <i>p</i> value | <i>T. va</i> ginalis +ve | p value | Co-infection | <i>p</i> value |
|--------------|--------------------|--------------------|----------------|-----------------------------|---------|--------------|----------------|
| 15-20 | 167 | 31 | 0.13 | 13 | 0.51 | 5 | 0.79 |
| 21-30 | 661 | 187 | 0.37 | 46 | 0.70 | 21 | 0.46 |
| 31-40 | 317 | 84 | 0.84 | 14 | 0.23 | 6 | 0.68 |
| 41-50 | 83 | 12 | 0.07 | 5 | 0.05* | 0 | 0.54* |
| 51-60 | 12 | 5 | 0.37* | 2 | 0.08* | 0 | 0.07* |
| Total | 1240 | 319 | | 80 | | 32 | |
| | | | *Fisł | ner-Exact test | | | |

 Table 1. Relationship between Age and Prevalence of VVC and TC

Table 2. Relationship between Risk factors and Prevalence of VVC and TC

| Risk factor | No. of subjects | C. albicans +ve | p value | <i>T. vaginalis</i> +ve | p value | Co- infection | p value |
|--------------------|--------------------|--------------------|------------|----------------------------|------------|------------------|------------|
| Pregnancy | 267 | 92 | 0.04 | 25 | 0.18 | 5 | 0.66* |
| Diabetes Mellitus | 167 | 67 | <0.01 | 19 | 0.04 | 13 | <0.01 |
| Oral contraception | 79 | 13 | 0.18 | 9 | 0.12 | 5 | 0.07* |
| Others | 727 | 147 | 0.13 | 27 | 0.01 | 9 | 0.05 |
| Total | 1240 | 319 | | 80 | | 32 | |

*Fisher-Exact test

| Occupation | No. of subjects | <i>C. albicans</i> +ve | <i>p</i> value | <i>T. va</i> ginalis +ve | <i>p</i> value | Co- infection | p value |
|---------------|--------------------|---------------------------|----------------|-----------------------------|----------------|------------------|------------|
| Housewife | 324 | 33 | 0.08 | 14 | 0.04 | 3 | 0.10* |
| Civil servant | 284 | 24 | 0.34 | 6 | 0.37 | 2 | 0.07* |
| Business | 149 | 17 | 0.03 | 6 | 0.36 | 3 | 0.48* |
| Student | 321 | 178 | <0.01 | 38 | <0.01 | 19 | <0.01 |
| Others | 162 | 67 | 0.45 | 16 | 0.14 | 5 | 0.61* |
| Total | 1240 | 319 | | 80 | | 32 | |

Table 3. Relationship between occupation and Prevalence of VVC and TC

*Fisher-Exact test

Table 4. Relationship between Marital Status and Prevalence of VVC and TC

| Marital status | No. of subjects | C. albicans +ve | p value | <i>T. va</i> ginalis +ve | p value | Co- infection | p value |
|-------------------|--------------------|--------------------|---------|-----------------------------|---------|------------------|---------|
| Single | 458 | 208 | <0.01 | 61 | <0.01 | 22 | 0.03 |
| Married | 730 | 99 | 0.12 | 15 | 0.09 | 8 | 0.054 |
| Co-habiting | 37 | 9 | 0.53 | 4 | 0.31* | 2 | 0.27* |
| Divorced | 15 | 3 | 0.48* | 0 | 0.39* | 0 | 0.68* |
| Total | 1240 | 319 | | 80 | | 32 | |

*Fisher-Exact test

4. DISCUSSION

Vulvovaginitis is a common problem globally and affects women of all socio-economic groups.

The present study observed a vulvo-vaginal candidiasis prevalence of 25.7%, similar to the 22.1% to 24.4% obtained in South-Western Nigeria [28,29], but lower than the 29.1% to 42% observed in Northern Nigeria [30,31]. Regional differences and sampling methods may be responsible for the disparity in the incidence of VVC recorded. Only *Candida albicans* species were isolated from the samples processed in the present study, in agreement with most studies in Nigeria [28-33]. Only one study has identified other Candida species in Nigeria [34].

A prevalence of 40.1% VVC was observed among the diabetics in our study, which is similar to the 46% observed in India [9].

In this study, 34.5% of the pregnant women were positive for *C. albicans*, which is higher than the 26% obtained among pregnant women in Ibadan, Nigeria [33], but still lower than the 62.6% observed in Eastern Nigeria [32].

This study observed no association between age and *C. albicans* infection, the results were similar

to other studies where the highest prevalence was in the third decade of life [14,33,34]. Diabetes mellitus, pregnancy, beina а student and being single were significantly associated with C. albicans infection, in keeping with findings from other studies [32, 34-36]. Women with diabetes who have poorly controlled blood sugar levels are at greater risk of yeast infections as the increased glucose environment in the vagina lowers the vaginal pH, a situation which favours the growth of C. albicans. Similarly, pregnancy increases the risk for C. albicans infection because the increased concentration of reproductive hormones during pregnancy leads to an increase in the glycogen content of the vaginal epithelial cells thus providing a nutrient source for candida species [6,27,37,38]. Students are likely to be Single ladies and are more likely to be sexually active and more likely to engage in unsafe sexual practices. Although yeast infections are not considered sexually transmitted infections, sexual contact can spread the candida fungus.

Sexual transmission of VVC can occur during vaginal intercourse and receptive oro-genital sexual intercourse [11,13]. However this study is limited by our inability to explore sexual practices of the study population. Self-diagnosis of VVC should be actively discouraged. A study found that two-thirds of women who made a self-diagnosis of VVC were wrong [39]. The full implications of misdiagnosis/ mistreatment are not fully understood, but could cause a delay in the diagnosis and treatment of some more serious underlying pathology. Also, resistance to antimycotics by Candida species may increase due to lack of discrimination in the use of these drugs.

The association between oral contraceptive use and candidiasis in the literature is inconsistent [11,35,36,40-44]. In our study subjects, most of whom used low-dose oral contraceptives, *C. albicans* was not associated with oral contraceptive use.

Infection with Trichomonas vaginalis is a serious public health problem which is not given necessary attention. The observed prevalence of vulvovaginitis due to T. vaginalis infection was 6.5%, close to the 5.2% obtained in the Niger Delta region of Nigeria [45], but lower than the 18.7% observed in South Eastern Nigeria [46] and higher than the 1.2% observed in North Central Nigeria [47]. Our observed prevalence of 9.4% T. vaginalis infection among pregnant women falls within 0.5% and 24% range observed in other studies in Nigeria [48-50]. These differences in prevalence rates could be due to regional factors, as prevalence rates have been noted to differ among different populations within the same country [51-54], and also between racial groups [19,55].

The present study found students to be more affected with T. vaginalis infection, different from the observation of other studies in Nigeria where traders/businesswomen were more commonly affected [46,49]. Our observation of a higher prevalence of T. vaginalis infection among single women, was similar to other reports in Nigeria [46]. Also, the observation of a higher prevalence in the third decade of life is in consonance with other local studies in Nigeria [45-47,56], but different from the fourth decade obtained in another study [50]. This finding is not surprising as students in their twenties are more likely to be single and engage in high risk sexual behavior including having multiple sexual partners and are therefore at a high risk for T. vaginalis infection.

Vaginal symptoms are not specific to VVC and *T. vaginalis* infections, but overlap with other vaginal infections. So symptoms and clinical

findings alone are unreliable for determination of the aetiology of the underlying problems. Diagnosis could be difficult using only microscopy, as about 50% of patients with culture-positive symptomatic VVC have negative wet mount microscopy [27], with vaginal culture presently the most accurate method for diagnosis of VVC. So, the diagnosis of VVC should combine clinical findings, microscopic examination and vaginal culture [27].

The incidence of trichomoniasis from different studies could be higher than obtained, considering the fact that the diagnosis of *T. vaginalis* infection is limited by low accurate diagnostic methods [57]. Wet mount microscopy used for diagnosis has a sensitivity of between 35% and 60% [58,59], meaning that many cases will remain undetected. Considering the inefficiency of diagnosis, our results may grossly underestimate the prevalence. The low sensitivity is thus an impediment to effective treatment of *T. vaginalis* infection. There is therefore a need for cheaper, more available and yet more efficient diagnostic tools for detection of *T. vaginalis* in resource-poor settings.

A high rate of re-infection has been observed in patients in whom trichomoniasis was diagnosed [60]. This might be related to the fact that *T. vaginalis* infection can persist for long periods in the female urogenital tract [61]. Untreated partners, therefore remain a source of re-infection and so treatment programmes should include partners.

T. vaginalis is not routinely screened for in most settings [4]. Also, there is an overlapping of the clinical manifestation of bacterial vaginosis, vulvo-vaginal candidiasis and trichomoniasis, making clinical diagnosis unreliable. Therefore, clinical guidelines which would include testing and treatment of partners should be developed and used, and should also include laboratory testing for all potential/common causes of vaginitis.

Limitations of this study include the fact that sexual practices could not be successfully explored, especially as *T. Vaginalis* is the most prevalent non-viral sexually transmitted infection globally. Newer strategies to extract such information need to be developed. The use of questionnaires, even when the subjects are helped to fill them has not been helpful. It has also been observed that self-reported data on sexual behaviour are not reliable [17]. We suggest that *Trichomonas vaginalis* infection become a reportable infection in Nigeria.

5. CONCLUSION

Symptomatic VVC and *T. vaginalis* infection were found to be common in our environment. Diabetes mellitus, pregnancy, being single and being a student were found to be significantly associated with *C. albicans* and *T. vaginalis* infection. More efficient but affordable and available diagnostic tools are needed in resource-poor settings. Clearly defined clinical guidelines for symptomatic vulvovaginitis management are needed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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