

Conventional Pap Smears for Identification of Infectious Organisms (*Trichomonas vaginalis*, *Gardnerella vaginalis* and *Candida albicans*) among Patients Attending University College Hospital, Ibadan.

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ABSTRACT

Aim: The aim of this study was to determine the prevalence of *Trichomonas vaginalis* (TV), *Candida albicans* (CA) and *Gardnerella vaginalis* (GV) among women who had Pap smear screening at the University College Hospital, Ibadan, Nigeria.

Methods: A total of 450 Pap smears were retrieved from the archives and re-examined under the microscope to check for the following organisms; *Trichomonas vaginalis* (Cannon ball cells), *Gardnerella vaginalis* (clue cells) and *Candida albicans*.

Results: It was revealed that 8(1.8%) were positive to CA; 2(0.4%) to TV while 27(6%) cases were positive to GV. A case of GV and TV co-infection and 3 cases of GV and CA co-infection were observed.

Conclusion: Pap smear screening can be used in the diagnosis of some infections. The age groups of 36-45 years and 46-55years had the highest prevalence rate of *G. vaginalis*; *C. albicans* and *T. vaginalis*.

Keywords: Pap smear, *Gardnerella vaginalis*, *Trichomonas vaginalis*, *Candida albicans*.

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INTRODUCTION

Cervico-vaginal infections are diseases that affect the feminine genital tract, majorly the vagina and the cervix caused by microorganisms [1], which often make them to seek out an obstetrician and gynaecologist. Cervico-vaginal infections arise as a result of alteration in a patient's natural vaginal balance making the environment prone to proliferation of excessive fungi, bacteria and parasites. Women with untreated or mishandled cervico-vaginal infections are more likely to develop subsequent ovarian, tubal and/or uterine infections and eventually infertility [2]. Bacterial vaginosis, candidiasis, and trichomoniasis are responsible for 90% of the cases of the infectious origin and have been associated with premature labor, preterm delivery, low birth weight, increase prenatal mortality as well as predisposing them to HIV/AIDS [3] and cervical cancer [4]. Papanicolaou (Pap) test is a painless procedure to recover cells from the uterine cervix and is also used for the detection of cervical abnormalities and precancerous dysplastic changes, thus, serving as a screening test for the prevention of cervical cancer [5], the current Bethesda grading system of reporting pap smears is specifically designed to identify precursor lesions and malignancy, this system also see the importance of stating findings about microbial or parasitic infection [6], giving the need for identification of these organisms (*Trichomonas vaginalis*, *Gardnerella vaginalis* and *Candida albicans*). Bacterial vaginosis is best described as a disturbance of the vaginal microbial flora. This is characterized by a decrease or a substitution of the protective *Lactobacillus* species by an excessive proliferation of other anaerobic bacteria [1]. Cytologically, *Trichomonas vaginalis* is a

pear shaped or oval cyanophilic organisms; three dimensional clusters of neutrophils may be seen. *Candida albicans* is budding yeast (3-7mm) and pseudohyphae is present [7], *Gardnerella vaginalis* has a cytomorphological features of clue cells. Clue cells are squamous cells covered by coccobaccilli [8]. Microorganisms present in the vagina are determined by factors that alter their continued existence, including vaginal pH, quantity of glycogen for bacteria metabolism that helps the colonization of pathogenic microorganisms [9]. There are several external factors that make the vaginal microbiota susceptible to pathogenic microorganisms, such factors are: menopause, use of vaginal shower, Intra Uterine Device (IUD), tight clothes, use of internal absorbent (that causes bacteria proliferation when used more than recommended), use of wide spectra antibiotics, wrong hygiene habits, period, trauma after sexual act and unprotected sexual relationships [10].

MATERIALS AND METHODS

Study Area (Site)

This study was carried out in Cytology Laboratory, Department of Pathology, University College Hospital, Ibadan, Nigeria.

Ethical clearance

The protocol for this study was approved by the Ethics and Research Committee, College of Medicine, University of Ibadan, Ibadan, with the approval number UI/EC/20/0199.

Study Sample

A total of 450 Pap smear slides of year 2019 were retrieved from the Archive of Department of Pathology, University College Hospital, Ibadan, examined under the microscope at low and high power objectives for the presence of *Trichomonas vaginalis*, *Gardnerella vaginalis* and *Candida albicans*.

Staining procedures for Papanicolaou stain {Adopted from Ref. [11]}

Smears brought from the Clinic that were still in the fixative (95% Methanol) were hydrated in decreasing grades of alcohol and then water. Hydrated smears were stained in Harris haematoxylin for 4 minutes, rinsed in tap water, differentiated in 0.5% acid

alcohol briefly, rinsed in water again and blued in tap water for 10 mins. Stained Smears were rinsed in 70% alcohol, 90% alcohol for 10 sec and stained in Orange G6 for 2 min. Smears were rinsed in 2 changes of 95% alcohol, stained in Eosin Azure 50 for 4 min and rinsed in 2 changes of 95% alcohol briefly. Stained smears were dehydrated in 2 changes of absolute alcohol, cleared in xylene and mounted with DPX. The stained slides were examined under the microscope at low and high-power objectives for the presence of infectious micro-organisms.

Statistical Analysis

Data analysis was done using IBM-SPSS version 25.0. Data was summarized with frequency, proportions for categorical variables and Chi-Square were used to compare the mean and standard deviation of the different analytes at $P < 0.05$ statistical significance.

RESULTS

Table 1.0: Distribution of subjects According to their Age Groups.

Age (years)	Frequency (%)	Mean \pm SD
16-25	23(5.1)	45.28 \pm 11.67
26-35	74(16.4)	
36-45	129(28.7)	
46-55	127(28.2)	
56-65	77(17.1)	
66 and above	20(4.4)	
Total	450 (100)	

Table 2.0: Prevalence of *C.albicans*, *T.vaginalis* and *G.vaginalis* among subjects that had Pap smear examinations in 2019

Organisms	Number tested	Positive (%)	Negative (%)	P-value
<i>Candida albicans</i>	450	8(1.8)	442(98.2)	0.0001
<i>Trichomonas vaginalis</i>	450	2(0.4)	448(99.6)	
<i>Gardnerella vaginalis</i>	450	27(6)	423(94.0)	

P<0.0001

Table 3.0: Prevalence of *Candida Albicans* According to Age Groups

Age (years)	Number Tested (%)	Positive (%)	Negative (%)	P-value
16-25	23 (5.1)	0 (0)	23(5.1)	0.676
26-35	74 (16.4)	1(0.2)	73(16.2)	
36-45	129 (28.7)	4 (0.9)	125 (27.8)	
46-55	127 (28.2)	2 (0.4)	125 (27.8)	
56-65	77 (17.1)	1 (0.2)	76 (16.9)	
66 and above	20 (4.4)	0 (0.0)	20 (4.4)	
Total	450 (100)	8 (1.7)	442 (98.3)	

P>0.05

Table 4.0: Prevalence of TV According to Age Groups

Age(years)	Number tested (%)	Positive (%)	Negative (%)	P-value
16-25	23 (5.1)	0 (0.0)	23 (5.1)	0.910
26-35	74 (16.4)	0(0.0)	74 (16.4)	
36-45	129 (28.7)	1 (0.2)	128 (28.4)	
46-55	127 (28.2)	1 (0.2)	126(28)	
56-65	77 (17.1)	0 (0.0)	77 (17.1)	
66 and above	20 (4.4)	0 (0.0)	20 (4.4)	
Total	450	2 (0.4)	448 (99.6)	

P>0.05

Table 5.0: Prevalence of GV According to Subjects' Age Groups

Age (years)	Number tested (%)	Positive (%)	Negative (%)	P-value
16-25	23(5.1)	3(0.7)	20(4.4)	0.615
26-35	74(16.4)	5(1.1)	69(15.3)	
36-45	129(28.7)	7(1.6)	122(27.1)	
46-55	127(28.2)	8(1.8)	119(26.4)	
56-65	77(17.1)	4(0.9)	73(16.2)	
66 and above	20(4.4)	0(0.0)	20(4.4)	
Total	450(100)	27(6.1)	423(93.9)	

P>0.05

Table 6.0: Distribution of Mixed Cervico-vaginal infections According to their Age Groups

Age group	Number tested	TV and GV	GV and Candida
16-25	23	0	0
26-35	74	0	1
36-45	129	1	1
46-55	127	0	1
56-65	77	0	0
≥66	20	0	0
Total	450	1	3

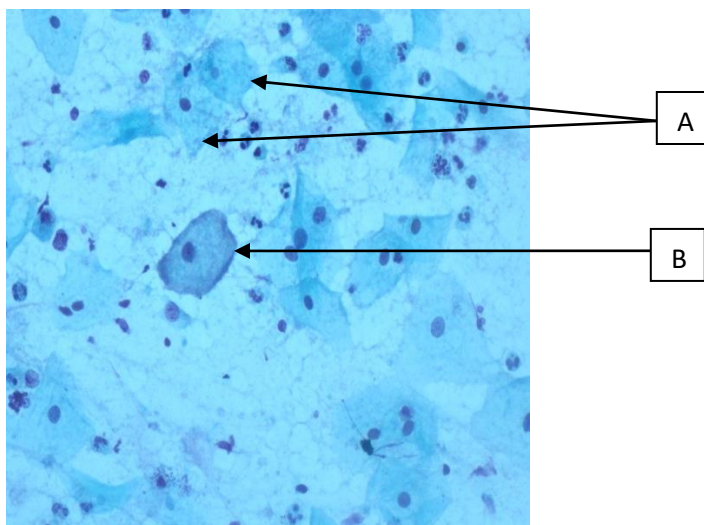


Plate 1.0: Pap smear from a woman (36-45years old) showing [A] superficial squamous cells [B] clue cells (X100)

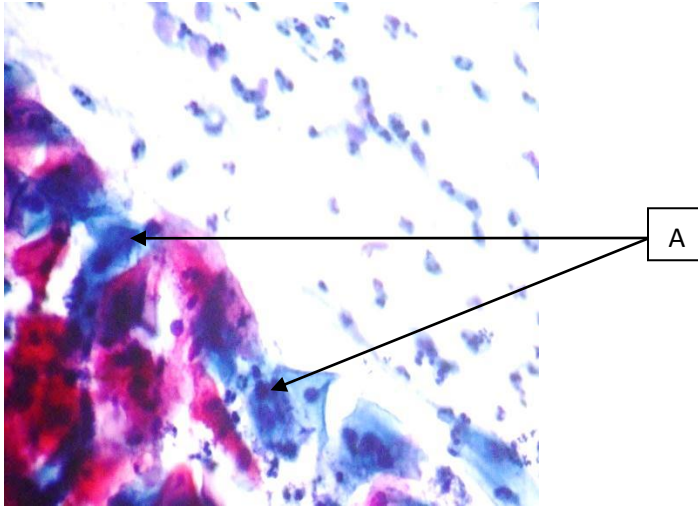


Plate 2.0: Pap smear from a woman (46-55years old) showing [A] clue cells (X100) .

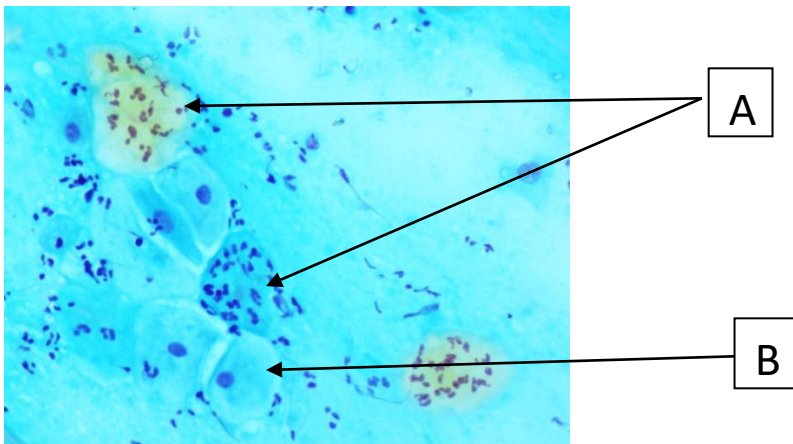


Plate 3.0- Pap smear from a woman (36-45years old) showing [A] cannon ball cells, [B] superficial cell, (X100)

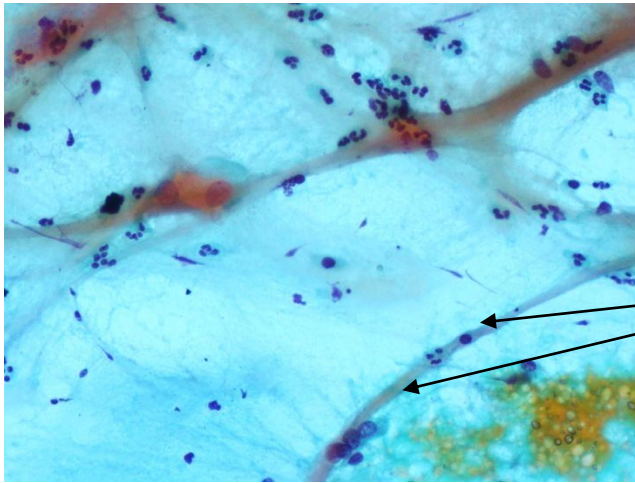


Plate 4.0- Pap smear from a woman (36-45years old) showing [A] Pseudohyphae of *Candida albicans* (X100)

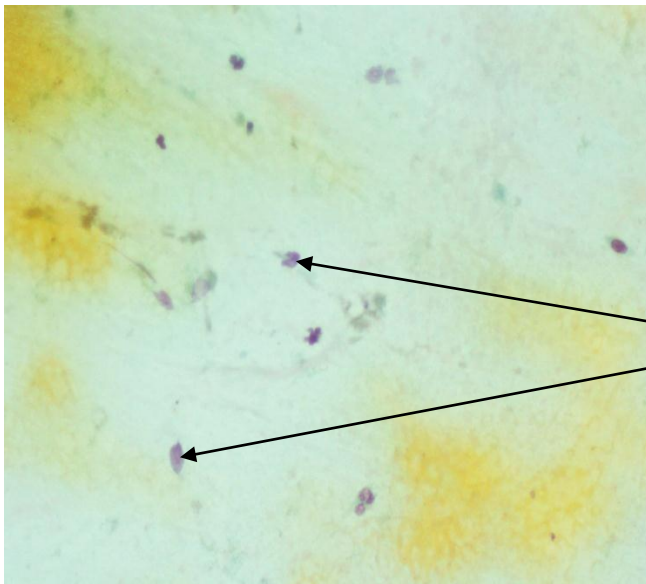


Plate 5.0: Pap smear from a woman showing [A] *Trichomonas vaginalis* (X100)

DISCUSSION

Cervicitis and vaginitis constitutes one of the most common problems in clinical medicine, and it is one of the reasons that lead women to seek out an Obstetrician and Gynaecologist [4]. Bacterial vaginosis, Trichomoniasis and Candidiasis are responsible for 90% of the cases of infectious origin. Papanicolaou test is a quick and painless procedure commonly used for diagnosing precancerous and cancerous lesions as well as cervico-vaginal infections [12].

About 450 Pap smear slides of women who attended UCH, Nigeria for cervical screening test for the year 2019 were retrieved for this study, of which 23(5.1%) of the subjects were between the ages of 16-25 years, 74(16.4%) were between the ages of 26-35years, 129(28.7%) were between the ages 36-45years, 127(28.2%) were between the ages 46-55years, 77(17.1%) were between the ages 56-65 years and 20(4.4%) were 66years and above. The mean age and standard deviation are 45.28 ± 11.67 .

This study revealed generally the prevalence of cervico-vaginal infection rate among women to be 8.2%. This result was obtained by conventional Pap smears testing. This finding is in agreement with Kalantari et al. [7] who reported a low bacterial vaginosis infection rate of 7.5% among women who attended a clinic that was supported by Cancer Patients Aid Association, Babol, Iran for cancer screening. According to this study, *Gardnerella vaginalis* had a prevalence of 6.0% being the highest, followed by *Candida albicans* (1.8%) and *Trichomonas vaginalis* (0.4%). This finding is in tandem with Alves et al. [8], where *G.vaginalis* has the highest prevalence (13.52%), *Candida albicans* (10.61%) and *T. vaginalis* (1.7%). *Candida albicans*

infections have also been diagnosed in Pap Smears from the cervix [10]

The infection caused by *Gardnerella vaginalis* is considered to be of sexually transmitted [11], although this hypothesis is refuted by its presence in virgins. This type of disease has been associated with lack of proper sexual orientation, education, hygiene habits, early onset of sexual activity and multiple partners [13;14]. On the other hand, the disequilibrium in the vaginal ecosystem, due to the rise in pH above 4.5 and a decrease or absence of colonization by lactobacilli, decrease of vitamin A, folate and calcium may be one of the predisposing factors to the proliferation of this micro-organism [15;16].

If *Gardnerella Vaginalis* which constitutes Bacterial Vaginosis is left untreated, it can lead to serious complications. Bacterial Vaginosis has been implicated in preterm delivery of low birth weight infants due to chorio-amnionitis, pelvic inflammatory disease (PID). Since up to half of the women with Bacterial Vaginosis are asymptomatic, the cervical cytology may be the only means of diagnosis [2]. Another important complication of BV as cervical dysplasia was explained by Tokyo et al. [17] and it was hypothesized that BV could be important in developing cervical neoplasia. In this condition, abnormal microflora produces carcinogenic nitrosamines which can lead to cervical cancer.

According to Maciei et al., [18], the presence of *Trichomonas vaginalis* can also be verified on Pap smear test [19]. Its infection is considered sexually transmitted, which risk factors are the number of sexual partners, socio-economic status, personal hygiene, or their association with other sexually transmitted disease. In pregnant women, it can cause rupture of membranes and premature labour [20].

This study revealed that subjects between the age groups of 36-45 years and 46-55years recorded the highest prevalence rate of *G. vaginalis*; *C. albicans* and *T. vaginalis*. The highest prevalence observed among these age groups could be due to the use of contraceptive devices such as intrauterine device, menopause, oral hormonal contraceptive of high dosage and unprotected sexual relationships.

The mixed infection in which there are two or more potentially pathogenic microorganisms was also observed in this study, 1(0.2%) case of TV and GV, 3 (0.6%) cases of GV and CA. Kumar *et al.*, [21] found higher incidence of co-infection in his study, also in a study carried out by Pathak *et al.*, [22], there was 2.8% cases of co-infection in his study. But there was only 0.9% case of co-infection in this study. A study by Gatski *et al.* (2016) reported that TV and BV are the common infections found in Human Immunodeficiency Virus (HIV) positive women. It was also revealed that infection with TV may create an environment favoring the development of BV.

CONCLUSION

This study has demonstrated that these organisms (TV, GV and CA) are found to be highest in women within the age groups of 36-45years and 46-55years. GV was the most predominant organisms observed. Routine Pap smear examination plays a significant role in the identification of cervico-vaginosis infections in asymptomatic women. Pap smear screening could be advantageous in early disease diagnosis and also helps in preventing further complications by commencing appropriate treatment.

REFERENCES

1. Fajardo EF, Braz GR, Rosa M, Marques T, Gomes BBM. Prevalence of Vaginal Infections in Women under Screening to HPV infection and cervical lesions in Brazillian Basic Health Service. *Int Arch Med Microbiol.* 2018; 1:006-008.
2. Godoy-Vitorino F, Romaguera J, Zhao C, Vargas-Robles D, Ortiz-Morales G, Vázquez-Sánchez F, Sanchez-Vázquez M, De la Garza-Casillas M, Martinez-Ferrer M, White JR, Bittinger K, Dominguez-Bello MG, Blaser MJ. Cervicovaginal Fungi and Bacteria Associated With Cervical Intraepithelial Neoplasia and High-Risk Human Papillomavirus Infections in a Hispanic Population. *Front. Microbiol.* 2018; 9:2533-2540.
3. Gatski M, Martin DH, Kissinger P. Co-occurrence of *Trichomonas vaginalis* and bacterial vaginosis among HIV-positive women. *Sex Transm Dis.* 2011; 38(3): 163-166.
4. Turovskiy Y, Sutyak NK, Chikindas ML. The aetiology of bacterial vaginosis. *J Appl Microbiol.* 2011; 110(5):1105-28.
5. Ochei J, kolhatkar A. *Medical Laboratory Science Theory and Practice 2000* Tata M,Graw- Hill Publishing Company Limited. Pp. 515 – 517.

6. Verma I, Jain V, Kaur T. Application of Bethesda System for Cervical Cytology in Unhealthy Cervix. *J Clin Diagn Res.* 2014; 8(9): OC26–OC30.
7. Kalantari N, Salman G, Masomeh B. *Trichomonas vaginalis*, and *Gadnerella* in cervical smears of Iranian Women for cancer screening. *North American Journal of Medical Sciences.* 2014; 6 (1): 25-29.
8. Alves JA, Nunes MD, Fakhouri R. Frequency of *Gadnerella vaginalis*, *Candida albicans*, *Trichomonas vaginalis* in pill users and copper intrauterine device users. *International medical society.* 2016; 9: 360-367.
9. Nguyen G, Smith B. *Essentials of Gyanaecologic Cytology.* 2011; Pg .18-20.
10. Avwioro OG, Olabiyi OE, Avwioro TO. Sensitivity of a Papanicolaou smear in the diagnosis of *Candida albicans* infection of the cervix. *North American Journal of Medical Sciences.* 2010, 2(2): 97-99
11. Demba E, Morison L, Loeff S, Awasana AA, Gooding E, Bailey R, Mayaud P. West B. Bacterial vaginosis, vaginal flora patterns and vaginal hygiene practices in patients presenting with vaginal discharges syndrome in the Gambia, West Africa. *BMC infectious Diseases.* 2005; 5: 1-12.
12. Ajileye AB, EZE GI, Fasogbon SA. Cytology Analysis of Urine among Cigarette Smokers. *American Journal of Biomedical Sciences.* 2016; 8 (1), 56-67.
13. Siona S, Deepak MN, Suma N, Ranjitha S. Role of Papanicolaou Smear in the diagnosis of pathologic Flora in asymptomatic Patients in rural health care setup. *Journal Of Clinical and Diagnostic Research.* 2017; 11(10): 10-13.
14. Karani A, Vuyst H, Luchters S, Othigo J, Mandaliya K, Chersich MF, Temmerman M. The Pap smear for detection of bacterial vaginosis. *Int J Gynecol Obstet.* 2007; 98:20-23.
15. Neggers YH, Nansel TR, Andrews WW, Schwebke JR, Yu KF, Goldenberg RL. Dietary intake of selected nutrients affects bacterial vaginosis in women. *J Nutr.* 2007; 137: 2128-2133.
16. De La Calle IJ, De La Calle AJ. *Vaginosis bacteriana.* *Med Clin. J.* 2009; 133: 789-792.
17. Tokyol C, Aktepe OC, Cevrioglu AS, Altindis M, Dilek FH. Bacterial vaginosis: Comparison of Pap smear and microbiological test results. *Mod Pathol* 2004; 17: 857-60.
18. Maciel GP, Tasca T, Carli-de GA. Aspectos clínicos, patogênese e diagnóstico de *Tricomonas Vaginalis.* *J Bras de Patol Med Laborat.* 2004; 10:152-160.
19. Avwioro OG. Diagnosis of trichomoniasis in Pap smears; How effective is it? *European Journal of Experimental Biology.* 2011 1: 10-13

20. Bonfanti G, Gonçalves TL. Prevalência de Gardnerella vaginalis, Cândida spp. e Trichomonas vaginalis em exames citopatológicos de gestantes atendidas no Hospital Universitário de Santa Maria-RS. Rev Saúde. 2004; 36:37-46.
21. Kumar N, Singh P, Rudra S. Papanicolaou smear as a tool for detection of cervico-vaginal infections in a rural tertiary care centre of Northern India: Retrospective analysis: Ind J Obstet Gynecol Res. 2016; 3: 343-347.
22. Pathak R, Pradhan P, Pudasaini S, Marhajan S, Basnyat AS. Study of trichomonas vaginalis and bacterial vaginosis in pap smear at a tertiary health care centre of Nepal. Nepal medical College journal. 2020; 22(1-2): 8-12.