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Research Article

IDENTIFICATION OF CERVICO-VAGINAL PATHOGENIC MICROORGANISMS IN PAP SMEAR AMONG REPRODUCTIVE WOMEN, WADMEDANI, GEZIRA STATE, SUDAN, 2023

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Abstract

Introduction: Female genital tract infections are usually asymptomatic and difficult to diagnose; hence they tend to be neglected healthcare problems. The most common pathogenic microorganisms of the vagina and cervix include *Gardnerella Vaginalis*(GV), *Candida* species (CS), *Trichomonas Vaginalis* (TV), *Human Papilloma Virus* (HPV)*, herpes Simplex Virus*(HSV) *and Actinomyces* species. **Aim:** The study aimed at detecting the frequency of cervico-vaginal pathogenic microorganisms in conventional Pap smear among women attending *Wad Medani* obstetrics and gynecology teaching hospital and some private clinics during the period from February 2020 to January 2023. **Methodology:** This is a cross sectional studyincluding137 reproductive women came to the health care facilities and presented for gynecological consultation with deferent gynecological problems. A total of 134 Pap smears were prepared and examined under the microscope. Cytological results were reported according to the 2014 Bethesda system for reporting cervical cytology and then checked for the presence of pathogenic microorganisms. **Results:** From the 134adequate Pap smears, 23(17%) showed pathogenic microorganisms,(47.8%) were *Gardnerella Vaginalis*, followed by (39.1%) were *Candida* species,(8.7%) were *Trichomonas Vaginalis* and (4.3%)was koilocytic change of *Human Papilloma Virus.* **Conclusion:** Conventional Pap smear has a definite role in the detection of cervico-vaginal microorganisms rather than inflammatory conditions and other reparative changes. Detected microorganisms were most frequent among rural women within the age group 26-36years and the majority were complained of vaginal discharge.

Keywords: Pap smear, Gardnerella vaginalis, Trichomonas vaginalis, Candida albicans, Human Papilloma Virus, Sudan.

INTRODUCTION

Despite they are a treatable diseases, cervico-vaginal infections are still a persistent health problem mainly in lower income countries. The complaint is caused by several microorganisms and affect the female genital tract mainly the uterine cervix and the vagina and make them to attend gynecology clinic (1). *Gardnerella vaginalis* (GV), *Candida spp* (CS) and *Trichomonas vaginalis* (TV) are responsible for 90% of the cases of these infections. Bacterialvaginosis "shift in flora" which mainly constituted by *Gardnerella vaginalis* which is the most common cause of vaginal discharge in females. Bacterial vaginosis is a common, nonspecific cervico vaginitis and is often asymptomatic. Cytologically is characterized by the presence of clue cells "intermediate and superficial squamous epithelial cells covered by a layer of coco bacilli bacteria that obscures the cell membrane" on Pap smears (2,3). Candidiasis is the most common fungal infection of cervico-vaginal canal. The disease is caused by *Candida spp* with *Candida albicans* being reported as the most common type that causing cervico-vaginal candidiasis. Both yeasts and non-septated pseudohyphae can be seen on Pap smears (4). Trichomoniasis is the most common non-viral sexually transmitted infection worldwide.

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The manifestation is caused by *Trichomonasvaginalis* parasite "a facultative anaerobic protozoan flagellate without mitochondria or peroxisomes". TV is a motile organism that lives in a female's lower genitourinary tract; it can also live in the prostate and urethra of men (5). *Human papillomavirus* (HPV) is the second most common cause of cervical cancer worldwide. HPV can infect in vulvar, vaginal, and cervical tissues and can persist throughout a lifetime leading to cervical cancer and/or precancerous lesions (6). Cytology is a typical professionally recognized accepted medical tool by a community and outpatient. It is a simplest and at the same time most cost effective medical test. Pap smear is a simple, painless, rapid and noninvasive technique suitable for detection of cervical cancer, precancerous lesions and non-neoplastic conditions of the vagina and cervix, thus, it servers as cervical cancers screening tool (1,7). The technique was introduced by George Papanicolaou and his associate Herbert Traut in 1940s and when it was introduced, cervical cancer was the leading cause of death in women at that time (8).

METHODOLOGY

This was a cross-sectional study conducted at *Wad Medani*, Gezira state, central Sudan in the period from February 2020 to January 2023. The study included 134 women attended *Wad Medani* obstetrics and gynecology teaching hospital and some private clinics. Inclusion criteria included subjects being

married, non-pregnant and non-menstruating reproductive women between 15-49 years. Ethical approval was obtained from Ministry of Health, Gezira State and all participants signed an informed consent form for their participation in this study.

Pap smear collection

Cervical samples were collected by a consultant gynecologists using sterile, disposable, medium size, plastic speculum and cervical brush (cervical broom). The patient firstly was asked to lay in the lithotomy position. The speculum, lubricated with water then was inserted into vagina under good light. The cervical brush was introduced into the endocervical canal, while its shoulder was positioned on 3 o'clock position of the ectocervix, and the brush was rotated in a clockwise direction through 360° for 3 to 5 times in the same direction.

Pap smear preparation

The sample was smeared on a frosted glass slide and immediately fixed in 95% ethanol for 15 minutes and then stained with a Papanicolaou stain as a procedure described in 1960 by George Papanicolaou with some modifications. Smears were hydrated by using 90% alcohol, 70% alcohol, and water for 2 minutes for each step. Then the smears were stained by Harris haematoxylin for 5 minutes, differentiated in 0.5% hydrochloric acid alcohol for 10 seconds, blued in tap water for 10 minutes, dehydrated in 70% alcohol for 2 minutes and two changes of 95% alcohol for 2 minutes for each and then stained by Orange G.6 for 2 minutes, then washed in 95% alcohol and finally stained by EA50 for 2 minutes. Then, the smears were washed in 95% alcohol, cleared by xylene, mounted by DPX and covered by cover glass and allowed to dry. All slides were examined microscopically and the results were reported according to the 2014 Bethesda System for Reporting Cervical Cytology.

Data collection and analysis

Data were collected using a pretested and well-constructed questionnaire included all required information in addition to a written informed consent. The collected data were then analyzed using Statistical Package for Social Sciences (SPSS) software programme (version 20).

RESULTS

The study included a total of 134reproductive women of varying ages between 15 to49 years old attending *Wadmedani* Obstetrics and Gynecology Teaching Hospital and some private clinics. Half of the participants (45.5%) were between26 - 36 years old and the majority (59%) were rural residents. 69.7%of participants complained of vaginal discharge with odor or odorless while3.0% complained of abnormal/intermenstrual bleeding and 27.3% were apparently normal. From a total of 134conventional Pap smears, cervico-vaginal pathogenic microorganisms had been diagnosed in 23(17%) smears. Of these, *Gardnerella vaginalis*, *Candida spp*, *Trichomonas vaginalis* and koilocytic changes of HPV were detected on Pap smears with GV (47.8%)being the most frequent microorganism followed by fungal *Candida* infections(39.1%), TV (8.7%), and koilocytes of HPV detected in one smear (4.3%) [Table 1]. All these microorganisms were identified in Figure 1, 2, 3 and 4 respectively.

Table 1. Frequency of detected pathogenic microorganisms on Pap smearsof study subjects

Microorganism	Frequency	Percentage %
GV	11	47.8
CS	9	39.1
TV	2	8.7
HPV	1	4.3
Total	23	100

Table 2. Distribution of the detected pathogenic microorganisms according age groups

	Age			
Microorganism	15-25years	26-36years	>36years	Total
GV	0	9	2	11
CS	1	5	3	9
TV	0	1	1	2
HPV	0	0	1	1
Total	1	15	7	23

Table 3. Distribution of the detected pathogenic microorganisms according to residency

	Residence		
Microorganism	Rural	Urban	Total
GV	8	3	11
CS	6	3	9
TV	1	1	2
HPV	0	1	1
Total	15	8	23

Table 4. Prevalence of pathogenic microorganisms according to clinical presentations

	Clinical DX		
Microorganism	VD	Abn bleeding	Total
GV	10	1	11
CS	9	0	9
TV	2	0	2
HPV	1	0	1
Total	22	1	23

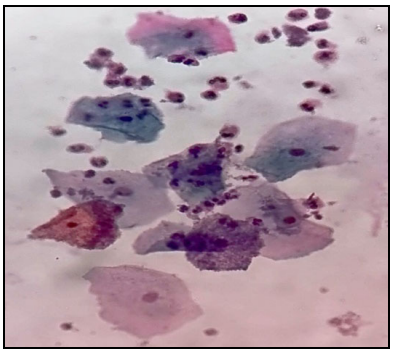

Figure 1. Pap smear showing intermediate and superficial clue cells of *Gardnerella vaginalis* (Pap; 40X).

Figure 2. Pap smear showing pseudohyphae and spores of *Candida spp* (Pap; 40X)

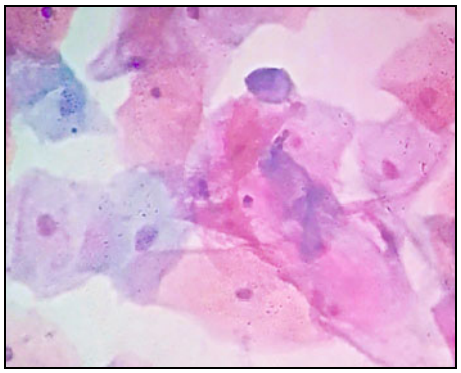

Figure 3: Pap smear showingtrophozoite form of *Trichomonas vaginalis* (Pap; 40X).

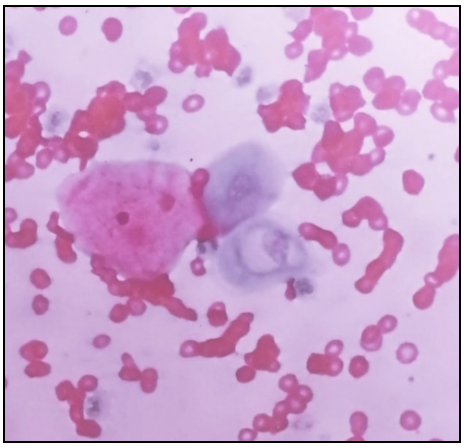

Figure 4: Pap smear showing Koilocytic change of *Human Papilloma Virus* (Pap; 40X).

DISCUSSION

Genital tract infections constitute important health problems worldwide. These infections are treatable and preventable, but if untreated, can lead to several complications such as pelvic inflammatory disease (PID), puerperal sepsis, preterm birth, infertility, and even to cervical cancer. Cervico-vaginal infections are frequently asymptomatic which is been difficult to diagnose and may be accompanied with several nonspecific symptoms like increased vaginal discharge and intermenstrual bleeding (9). Conventional Pap test is a frequently used,cost-effective method, for cervical cancer screening and early detection, diagnosis and even for prognosis and follow up. The technique isalso considered very effective in the detection of cervico-vaginal microorganisms especially in developing countries(10). Cytological diagnosis ofcervical and vaginal organisms iseither through identification of microorganism itself or through the detection of its cytological changes (11,12). In Sudan, cervical cancer remains a major public health problem with inadequate documentation and no current strategy for cancer elimination. There is no programme for HPV vaccination nor a national well-coordinated surveillance programmes. Numerous factors result in delaying cervical cancer care include poverty, health illiteracy and lack of awareness regarding cervical cancerearly symptoms and risk factors and Pap smear implications as a diagnostic tool. Therefore, cervical cancer usually discovered at late stages (13). Infections by cervico-vaginal microorganisms in the female genital tract and their associated inflammatory response maybe of diagnostic importance. Several studies reported apossible relationship between persistent infections and development of malignancies(14).

In the present study, cytological findings showed that detected microorganism infections included *Bacterial vaginosis, Candidiasis, Trichomoniasis* and HPV infections and the frequencies of these infections were beaked at reproductive-age group between 26 - 36 years in agreement with that reported by Sabu *et al.* and Peebles *et al.* (15, 16). The higher prevalence of cervico-vaginal infections among rural women in comparison to urban subjects was expected due to many reasons such as poverty, lack of awareness, illiteracy, poor sanitation, poor hygiene and lack of health education among Sudanese rural community in similar with that published by Jyoti (17)who reported a significant correlation of cervical diseases with rural environment. In Pap smear, GV was the most identified microorganism followed by CS,TV and HPV respectively. Clue cells of GV were noted in 11 reproductive women. That was in agreement with a study conducted by Pathak *et al.*(18) who observed that bacterial vaginosis was the most predominant type of cervico-vaginal infections. GV bacterium constitutes *Bacterial vaginosis* infection. Interestingly, GV existence in cervico-vaginal area may lead to decrease in protective vaginal lactobacilli, favoring HPV and other sexually transmitted infections(19). In addition, an enzyme sialidase that is produced by GV can degrade the vaginal mucosal surface that inhibit bacteria-host interactions increasing susceptibility to Human Papilloma Virus infection and malignant transformation. The risk may reach a twofold increased significant risk factor for low-grade cervical precancerous lesion (LSIL/CIN1) according to a previous study conducted by Cascardi *et al.* (20)

Candida spp were diagnosed in 9 Pap smears from rural resident complained of vaginal discharge. Microscopically, both fungal spores and pseudohyphae were detected, but pseudohyphal forms were more numerous than spores. That was in agreement with a study done by Misra *et al*(21) and reported that *Candida spp* and coco bacilli were the most commonly detected pathogens in the inflammatory smears among sexually active women. Pap smear can be used as a cheaper and shorter first step examination method for cervico-vaginal *Candidiasis*. In present study, *Candidiasis* is the most frequent fungal infection of cervix caused by *Candida spp*similar to a study conducted by Chibvongodze *et al.* (22). *Trichomonas Vaginalis* trophozoite was examined in 2Pap smears. TV is the most common protozoan sexually transmitted disease. As demonstrated from nationwide population database, TV has highly association with the development of cervical lesions. According to Kovachev (2020), TV may contributes to cervical cancer through different mechanisms, for example through changing the vaginal microbiota or it may contributes as a co-factor with other sexual transmitted diseases such as HPV infection (23). Koilocytic changes of HPV were identified in 1Pap smear of woman with age more than 36 years old and complained of abnormal intermenstrual bleeding. HPVs are a group of more than 221 genotypes DNA viruses categorized in to low-risk and high-risk groups according to their risk to cervical cancer development. Many studies reported that about 99.8% of cervical cancers and 70% of invasive carcinomas have a high-risk HPV mainly HPV 16 and HPV 18 genotypes (6,20). The prevalence of HPV infection is higher among sexually active women (70% of patients over 25 years old and this was in agreement with our study (24). Most infections usually regress spontaneously, with or without manifestations of dysplasia; but, in some cases HPV infection can persist and develop to cervical cancer(25).

Conclusion

Conventional Pap smear is an effective method for detection of cervico-vaginal microorganisms. This technique is simple, cost effective and suitable and it is applicable in lower income countries. *Gardnerella Vaginalis* was the most frequently identified organism followed by *Candida spp*. *Trichomonas Vaginalis*, and *Human Papilloma Virus* were seldom noted. Cervico-vaginal microorganisms were higher frequent among rural women within age group 26-36years.Well-coordinatedopportunistic screening programmes involving all ladies with abnormal vaginal discharge and or abnormal vaginal bleeding could be very helpful in early diagnosis of cervical diseases, may lead to down staging and prevention of cervical cancer development.

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Conflict of interest: Have nothing to declare.

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