**PREVALENCE OF BACTERIAL VAGINOSIS AND HIGH RISK HPV CO-INFECTION AMONG HIV-POSITIVE WOMEN AT MACHAKOS LEVEL-5 HOSPITAL, MACHAKOS COUNTY, KENYA**

**Abstract**

**Background:** Cervical cancer is a huge health burden globally. Precancerous cervical lesions, if not diagnosed and treated early enough, could become cervical cancer. HIV-positive women are among the highly susceptible individuals to both bacterial vaginosis and cervical cancer due to their compromised immune systems. In Kenya, cancer of the cervix is the second most common type of cancer among women. HIV Infected women have a high prevalence of HPV infection and associated cervical lesions. The Human Papilloma Virus (HPV) sub types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 are attributed to causing cervical cancer, with subtypes16 and 18 being the most common. Bacterial vaginosis is a type of bacterial inflammation due to an imbalance of harmful and beneficial bacteria in the vagina. Women with bacterial vaginosis infection could easily acquire HIV, among other sexually transmitted infections. This is because bacterial vaginosis infection leads to disruption of the normal vaginal micro flora, which results in increased vaginal PH. This, therefore, results in the recruitment of the immune cells to the HIV infection target cells.

**Objective:** The study aimed to determine the prevalence of bacterial vaginosis and high-risk HPV co-infection among HIV-positive women at Machakos Level-5 Hospital in the Comprehensive Care Clinic.

**Study Design:** The study used a prospective cross-sectional design.

**Place and Duration of Study:** Comprehensive Care Clinic (CCC), Machakos Level 5 Hospital, between July and October 2023.

**Methodology:** The study included 125 HIV-positive women aged 21 to 64 years who consented to take part in the study. The participants were screened for high-risk HPV sub types using real-time PCR and for cervical lesions and bacterial vaginosis through microscopic examination of pap smears.

**Results:** 125 HIV-positive women aged between 21 and 64 years were included in the study. Out of the 125 study participants, 36 tested positive (28.8%) for various HPV sub types. 13(10.4%) study participants out of the 125 tested positive for bacterial vaginosis. 11(8.8%) study participants had bacterial vaginosis and HPV co-infection.

**Conclusion:** The prevalence of bacterial vaginosis and HPV co-infection among HIV-positive women at Machakos Level 5 Hospital is 8.8%. Of all the risk factors analyzed, only method of contraceptive used was found to have a significant association with bacterial vaginosis and HPV co-infection (P = .002).

*Keywords: Cervical cancer,HIV positive women, bacterial vaginosis, Human Papilloma Virus, Pap smear*

1. **INTRODUCTION**

Increased incidence of cancer of the cervix is a rampant health issue globally. Cancer of the cervix is ranked as the fourth leading type of cancer among women globally, according to data published by the World Health Organization in 2022. The incidence of cervical cancer in 2022 was 662,301, which is 6.9% of the total number of cancer incidences among females globally, with 348,874 succumbing to the disease [1]. In Africa, cervical cancer is the second most common type of cancer; the incidence was 18.5% of the total cancer incidences among females. In Kenya, in the year 2022, cervical cancer was estimated to be 5845 new cases, representing 20.6% of the total number of all new cases of cancer in Kenya among women. It is also a great contributor to deaths among women in Kenya as 3591 women lost their lives [2].

HIV-positive women are at risk of developing many infections, including bacterial vaginosis and cervical lesions. limited access to quality health care systems and social cultural barriers inhibit early and comprehensive diagnosis of cervical lesions and treatment, which often leads to the development of cervical cancer among these patients. Since bacterial vaginosis and HPV co-infection are huge problems among HIV-positive women and could likely trigger cervical cancer development, it is therefore vital to carry out HPV and Pap smear tests among HIV-positive women of childbearing age [3]. This helps determine if they have high-risk HPV sub types and reveals the relationship between bacterial vaginosis and high-risk HPV co-infection with cervical lesions.

Precancerous cervical lesions can progress to cervical cancer if not treated early enough. Sexually transmitted Human Papillomavirus has majorly been attributed to causing cervical lesions [4]. HPV is a non-enveloped DNA virus. There are several HPV sub-types but the High-Risk Human papillomavirus sub types, including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. Most HPV sub-types do not lead to cancer but the high risk ones have a great potential to cause cervical cancer in women especially for HPV sub-type 16 and 18. Cervical cancer may take twenty or more years to develop after an HPV infection. It is therefore important that women are screened regularly.

Bacterial vaginosis is a type of bacterial inflammation due to an imbalance of harmful and beneficial bacteria in the vagina. This disease presents with symptoms like an itchy vagina, fishy vaginal odour, and green, white, or grey discharge. Previous research has indicated a correlation between bacterial vaginosis and high-risk HPV. Bacteria like *Gardnerella spp*., *Mobilincus spp., Megaspahera spp., Sneathea spp. and Prevotella spp.* whose overgrowth cause bacterial vaginosis have been indicated to be in large numbers in women positive for High-Risk HPV[5].

Human Immunodeficiency virus infection (HIV) is still a health burden in African countries. Kenya has an HIV epidemic, recording a prevalence of 3.3% in the year 2024, according to the World Health Organization. Women who have bacterial vaginosis infection could easily acquire HIV, among other sexually transmitted infections. Research indicates that bacterial vaginosis is linked with a high risk (60%) of acquiring HIV-1 in women. This is because bacterial vaginosis leads to disruption of the normal vaginal micro flora, which results in increased vaginal PH. This, therefore, results in the recruitment of the immune cells to the vagina, which eventually become the HIV infection target cells [6].

High-risk HPV infection has been highly correlated with cervical lesions and cervical cancer especially in HIV positive women [18]. HIV positive women are likely to develop cervical cancer six times more than HIV negative women according to World Health Organization 2024. Bacterial vaginosis has been attributed to an increase in the risk of the development of both cervical lesions and cervical cancer, as shown by the studies carried out [16]. However, the role of bacterial vaginosis and high-risk HPV co-infection in the development of cervical lesions is still elusive. Additionally, numerous studies have evaluated the prevalence and impact of BV and HPV individually, but fewer have focused on their co-infection, particularly in HIV-positive women and specifically from Kenyan settings, justifying the need for this study.The study also aimed at revealing the prevalence of bacterial vaginosis and HPV co-infection among HIV-positive women. The findings from this study shared with the policymakers in the health sector and specifically the comprehensive care clinic are beneficial in making informed decisions in managing HIV-positive women, leading to improved healthcare quality.

1. **MATERIALS AND METHODS**
   1. **Study Design**

A prospective cross-sectional research design was used to carry out the research. 125 HIV-positive women at Machakos Level 5 Hospital attending the Comprehensive Care clinic who consented to take part in the study and met the inclusion criteria were screened for high-risk HPV sub types using real-time PCR and had cervical lesions and bacterial vaginosis examined using the pap smears taken. Their demographic data was collected using questionnaires.

**2.2 Collection of the Cervical Samples**

Before the sample collection, the study participants were educated on the benefits and the sample collection process. After informed consent and taking of their demographic data, they were taken in a secluded room and put in a comfortable position. A single-use sterile speculum lubricated using KY gel was inserted in the vagina to widen the vaginal walls for easy access and visualization of the cervix. Scrapping of both the ectocervix and the endocervix was done using a cyto brush, a pap smear made on a clean glass slide labeled with the study participants' details and put in a 95% ethyl alcohol fixative. The cyto brush was then vigorously stirred in a cartridge containing the HPV fixative and labeled with the study participants' details

like the unique hospital number and date of collection, they were then inserted in an alcohol-based fixative and transported to the lab for screening.

**2.2.1 Processing of Samples for High-Risk HPV**

The Xpert HPV Assay for the detection of HPV sub types was performed on the Cepheid Gene Xpert Instrument system using Real-time PCR. The test was carried out in accordance to the manufacturer's instructions and following the standard operating procedures.

**2.2.2 Processing of the Pap Smear Samples**

The Pap smear samples were stained using the Papanicolaou staining procedure and observed under the microscope to screen for both cervical lesions and bacterial vaginosis.

2.2.2.1 Data analysis

The HPV DNA and Pap smear results were recorded in an Excel spreadsheet and then imported into SPSS software (IBM SPSS Inc. application software 29.0.2.0) to analyse dependent and independent variables. The results were tabulated to show the P-value proportions. A chi-square test was then done to determine if there was a significant association between bacterial vaginosis and HPV co-infection and the demographic data. A P value of less than 0.05 was considered statistically significant at a confidence interval of 95%.

1. **RESULTS**

125 HIV-positive women aged between 21 and 64 years were included in the study. Out of the 125 study participants, 36 tested positive (28.8%) for various HPV sub types. 13(10.4%) study participants out of the 125 tested positive for bacterial vaginosis. The number of study participants with bacterial vaginosis and HPV co-infection was 11 (8.8%).

Additionally, out of the 125 study participants, 34 tested positive for various HPV sub types. 87 tested negative while 4 had invalid results. Of the 34 positive HPV results, 4 were positive for HPV 16, 8 were positive for HPV 18-45 and 18 were positive for other high risk HPV sub types. 2 study participants tested positive for both HPV 16 and other high risk HPV sub types. 2 other study participants tested positive for HPV 16, HPV 18-45 and other high risk HPV sub types. Examination of cervical lesions revealed that 112 (89.6%) , were negative for intraepithelial lesions or malignancy while 12 study participants had abnormal smears.

Demographic data, which included age, education level, and marital status, were analyzed, and none was found to have any statistically significant association with bacterial vaginosis and HPV co-infection. Table 1 is a cross-tabulation of the age of the study participants and the bacterial vaginosis and HPV co-infection. Ages 41-50 had the most number of confections (45.5%), while study participants of ages 61-70 did not record bacterial vaginosis and HPV confection. There was no statistically significant association between age and bacterial vaginosis and HPV co-infection (P = .721) (Table 1). Among the 125 study participants, 17 (13.6%) were single, 55 (44%) were married, 23(18.4%) were divorced and 30 (24%) were widowed. There was no statistically significant association between marital status and bacterial vaginosis and HPV co-infection (P = .233) (Table 2). The education level of the study participants revealed that 35 (28%) had not gone to school. Most of the study participants 41 (32.8%) went up to the primary school level. There was no statistically significant association between education level and bacterial vaginosis and HPV co-infection (P = .087) (Table 2).

Other factors considered, like a history of previous Pap smear screening and sexual activity also did not have a statistically significant association with bacterial vaginosis and HPV co-infection. When assessing the family planning method used by the study participants, condoms were the most used method by 71 (56.8%) participants. Pills were the least used by 10 (8%) participants. There was a significant association between the family planning method used and bacterial vaginosis and HPV co-infection (P = .002) (Table 3).

**Table 1: Comparison of Age Versus Bacterial Vaginosis and HPV co-infection.**

**Bacterial Vaginosis and HPV Co-infection**

**Age(Years)** **Frequency**   **Positive** **Negative** **P Value**

21-30 19(15.2%) 2 17 .721

31-40 22(17.6%) 1 21

41-50 42 (33.6%) 5 37

51-60 38(30.4%) 3 35

61-70 4(3.2%) 0 4

Total 125(100%) 11 114

**Table 2: Comparison of Education Level, Marital Status Versus Bacterial Vaginosis and HPV co-infection.**

**Bacterial Vaginosis and HPV Co-infection**

**Education Level** **Frequency**   **Positive** **Negative** **P Value**

Not Gone 35 ( 28%) 5 30 .087

Primary 41( 32.8%) 4 37

Secondary 40 (32%) 2 38

College/University 9 (7.2%) 0 9

Total 125 (100%) 11 114

**Marital Status Frequency Positive Negative P Value**

Single 17(13.6%) 4 13 .233

Married 55(44%) 3 52

Divorced 23(18.4%) 2 21

Widowed 30(24%) 2 28

Total 125(100%) 11 114

**Table 3: Comparison of Family Planning Method, Sexually Active and History of Pap Smear Versus Bacterial Vaginosis and HPV co-infection.**

**Bacterial Vaginosis and HPV Co-infection**

**Contraceptive Method**  **Frequency**  **Positive Negative** **P Value**

Natural 17(13.6%) 6 11 .002

IUCD 5(14%) 0 5

Injection 22(17.6%) 1 21

Pills 10(8%) 0 10

Condom 71(56.8%) 4 67

Total 125(100%) 11 114

**Sexually Active**  **Frequency**  **Positive Negative** **P Value**

Yes 115(92%) 11 104 .310

No 10(8%) 0 10

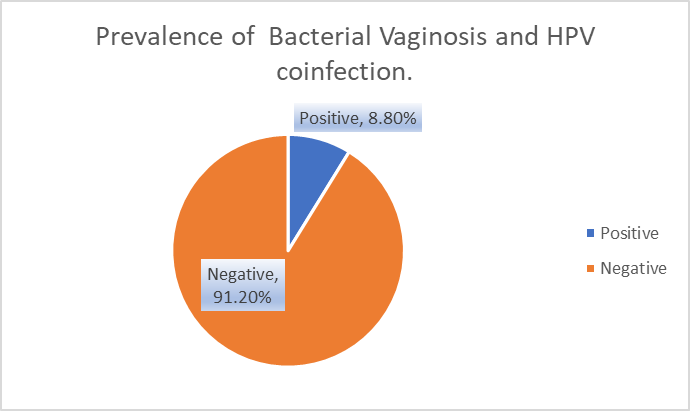
Total 125(100%) 11 114

**Ever Had Pap Smear**  **Frequency**  **Positive Negative** **P Value**

Yes 16(12.8%) 0 16 .186

No 109(87.2%) 11 98

Total 125(100%) 11 114



**Fig 1: Prevalence of Bacterial Vaginosis and HPV Co-infection.**

1. **DISCUSSION**

In the current study, the prevalence of bacterial vaginosis and high risk HPV co-infection was 8.8%. Out of these factors (age, marital status, educational level, history of previous pap smear screening, family planning method and sexual activity,) none but family planning method used were found to have statistically significant association with bacterial vaginosis and HPV co-infection.

The prevalence of our study is closer to the prevalence found in the study by Verteramo et al in Rome, Italy, which found out that the prevalence of bacterial vaginosis and HPV co-infection was 8.6% [7]. However, this study was done among women attending clinic for routine gynecological care and not specifically to HIV positive women. The prevalence of our study was slightly lower than that found in the study done by Guo et al. in 2012 which recorded the prevalence of bacterial vaginosis in High-risk HPV positive group at 11.2% [8]. Additionally, a meta-analysis of the relationship between vaginal microecology, human Papillomavirus infection and Cervical intraepithelial neoplasia by Liang et al. found the .prevalence of bacterial vaginosis in HPV positive group to be 13.4% [9]. A study in China showed a prevalence of bacterial vaginosis to be 5.9% in high risk HPV women aged between 20 -35 years [10].

Our study found no association between demographic factors like age, level of education, and marital status and bacterial vaginosis and HPV co-infection. This was in line with the research by Paesi et al., which found no significant association between social demographic features and HPV and lower genital tract infections [11]. Additionally, a study by Muia et al., at Machakos Level 5 Hospital among HIV Positive women, revealed no significant association between factors like contraceptive use,marital status and educational level with the prevalence of Bacterial vaginosis. Age was the only factor that showed a significant association with BV prevalence (17).

The study also found no significant association between sexual activity and previous Pap smear screening with Bacterial vaginosis and HPV co-infection. However, there was a significant association between contraceptive use and BV-HPV co-infection. Similarly, a study by Vinodhini et al., found a significant association as the use of contraceptives increased the risk of acquiring HPV infection [12]. Another study by Marks et al. in Thailand revealed an association between the long-term use of combined oral contraceptives and increased HPV prevalence [13]. A study by Gupta et al. also found that the use of spermicidal contraceptives could lead to acquiring of bacterial vaginosis among women as the contraceptives could cause alterations of the vaginal micro flora hence predisposing the women [14]. Another study by Kovachev showed a statistically significant association between the use of condoms, IUCD and bacterial vaginosis [15].

1. **CONCLUSION**

The prevalence of bacterial vaginosis and HPV co-infection among HIV-positive women at Machakos Level-5 Hospital in the Comprehensive Care Clinic was 8.8%. All the factors considered were found to have no significant association with BV-HPV co-infection apart from the contraceptive method used.

**CONSENT**

The authors affirm that all study participants were explained the research process, the benefits, and their rights and freely consented to take part in the study.

**ETHICAL APPROVAL**

The authors affirm that the study guidelines have been adhered to and approved by the National Commission for Science , Technology and Innovations (NACOSTI) number ( NACOSTI/P/23/23612).The study was also approved by the Kenyatta University Ethical Review Committee.

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