

**Original Research Article**

**A comparative study of Pap smear findings among HIV seropositive women and seronegative Population**

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**Abstract**

**Background:** HIV positive women are more likely than HIV negative women to have cervical dysplasia. Incidence of HPV-related dysplasia increases as immune function declines. This study focuses on the pap smear abnormalities and associated high risk behaviors between seropositive and seronegative women. **Methods :** The present hospital based a cross-sectional study was conducted in the Dept of Gynae and Obstetrics, Eden OPD, Medical College and Hospital, Kolkata, West Bengal, India between August 2022 to January 2023. All HIV Seropositive women, attending at Eden OPD during same period after fulfilling the inclusion criteria were included in the study. Statistical data were analysed by using Microsoft Excel and SPSS V.20 software. **Results :** Among seropositive women, 22%, 12% and 2% women tested to have LSIL, HSIL, SCC respectively. 32% women had inflammatory / NILM report. 84% of Seronegative women tested negative for any malignancy and 2(1.3%) patients had ASCUS report. (abnormal squamous cell of undetermined significance). 10%, 4%, 0.7% of the women without HIV reported LSIL, HSIL, SCC respectively.. Association of report in two groups was statistically significant ( $p < 0.0001$ ). **Conclusion :** HIV/AIDS is associated with a risk for cervical cytological abnormalities

and hence the need for periodic pap smear screening in this high risk group to reduce the global burden of cervical cancer and timely intervention at premalignant stage.

**Keywords :** Pap smear, seropositive, prevalence

**Introduction :**

Cancer of the cervix uteri is the 3rd most common cancer among women worldwide, with an estimated 569,847 new cases and 311,365 deaths in 2018 (GLOBOCAN). The majority of cases are squamous cell carcinoma followed by adenocarcinomas. HIV-1 is a retroviral infection that evolved ~120 years ago, and entered human populations >60 years ago via a number of chimpanzee/gorilla-to-human cross-species transmissions,<sup>1</sup> with the subsequent development of a worldwide pandemic.

The prevalence of squamous intraepithelial lesions (SIL) in HIV positive group varies from one study to another. Cervical cancer following cervical dysplasia is a major health concern. About 96,922 new cervical cancer cases are diagnosed annually in India (estimates for 2018). Cervical cancer ranks\* as the 2nd leading cause of female cancer in India. Cervical cancer is the 2<sup>nd</sup> most common female cancer in women aged 15 to 44 years in India.<sup>2</sup> Young women are the most affected by HPV and by multiple infections. The prevalence tends to decrease with increasing age.<sup>3</sup> A high viral load and the persistence of oncogenic HPV types are progression factors for precancerous lesions and cervical cancer.<sup>4</sup> Persons with HIV, even when effectively treated with ART have an increased risk and rate of HPV acquisition, more frequent carriage of multiple HPV types, and an increased rate of HPV-related disease including more rapid progression to malignancies.<sup>5</sup>

There are several risk factors associated with cervical cancer, including race, parity, smoking, the use of oral contraceptives, age of first sexual intercourse, multiple partners, immune deficiency, and low socioeconomic status.<sup>6-8</sup> In many societies, due to a range of social and legal discrimination issues, sex workers are usually poorly identified and do not have regular Pap smears or other routine health check-ups. Human immunodeficiency virus (HIV) seropositive patients are another high risk group for cervical cancer.<sup>9-10</sup>

A fundamental part of the increased risk is 'immunodeficiency virus associated' due to B- cell, T-cell and NK-cell dysfunction, persistent inflammation, and persistent mucosal epithelial abnormalities.<sup>11</sup> HIV-positive women have high progression rate from low grade to high grade

SILs or cancer and are more likely to experience recurrence after treatment.<sup>12</sup> It therefore follows that this high risk group of women should be offered more frequent cervical cytology screening.<sup>13-</sup>  
<sup>14</sup> The association between cervical cancer and HIV/AIDS is so strong that the former is an AIDS defining criterion<sup>21</sup> In addition, CD4 immunosuppression is associated with persistence of high-risk HPV types<sup>16</sup>, and appears to play an important role in controlling the growth of neoplastic cells as well as the disease progression. On extrapolation of this fact, there is possibility that low CD4 count favours higher grade of cervical premalignant and malignant lesion. This assumption has been supported by a few studies that showed increased detection of cervical lesions in women with low immune status as measured by low CD4 counts.<sup>17-19</sup>

The purpose of this present study was to compare Pap smear findings in HIV-infected seropositive women and uninfected women at Eden Hospital and to correlate the Pap smear abnormalities among HIV positive women with their immune status.

The present hospital based a cross-sectional study was conducted in the Dept of Gynae and Obstetrics, Eden OPD, Medical College and Hospital, Kolkata, West Bengal, India between August 2022 to January 2023. All HIV Seropositive women, attending at Eden OPD during same period after fulfilling the inclusion criteria were included in the study. Statistical data were analysed by using Microsoft Excel and SPSS V.20 software

### **Materials and Methods**

Present hospital based comparative analysis, cross sectional study was conducted in the Dept of Gynae and Obstetrics, Eden OPD, Medical College and Hospital, Kolkata, West Bengal, India between August 2022 to January 2023.

Demographic data, such as age, parity, marital and educational status, smoking frequency, drug consumption habits, number of sexual partners, contraception, age at first intercourse, and history of post-coital bleeding were recorded. Pap smear was done for all participants from the exocervix and endocervix using a plastic Ayres's spatula and cytobrush and the specimens were sent to a pathology centre. The results were reported according to the Bethesda system.

**Method of Data Analysis Plan :** Pap smear was performed for all participants from the exocervix and endocervix, using a plastic Ayres's spatula and cytobrush. The samples were sent to

institutional pathology lab and reported by a pathologist as per modified Bethesda system. The cytopathologist was blinded to the HIV status of patient

For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS 20.0. and GraphPad Prism version 5. Two-sample t-tests for a difference in mean involved independent samples or unpaired samples. Paired t-tests were a form of blocking and had greater power than unpaired tests. A chi-squared test ( $\chi^2$  test) was any statistical hypothesis test wherein the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true. Without other qualification, 'chi-squared test' often is used as short for Pearson's chi-squared test. Unpaired proportions were compared by Chi-square test or Fischer's exact test, as appropriate. p-value  $\leq 0.05$  was considered for statistically significant

**Ethical considerations-** Study was initiated after obtaining the informed consents from the participants and ethical clearance from the institutional ethical committee.

**Results**

**Table 1: Distribution of Mean Age, CD4 Count in Two Groups**

		Number	Mean	SD	Minimum	Maximum	Median	p-value
<b>Age (Years)</b>	HIV	50	41.5600	7.9338	27.0000	61.0000	40.0000	<0.0001
	Without HIV	150	33.7533	4.8396	27.0000	45.0000	32.0000	
<b>CD4 Count (cells/ microlitre)</b>	HIV	50	583.3000	260.5644	136.0000	1277.0000	542.0000	<0.0001
	Without HIV	150	1255.3067	127.4340	1090.0000	1480.0000	1208.5000	

In group-HIV, the mean of age (mean $\pm$ s.d.) of patients was 41.5600  $\pm$  7.9338 years with range 27.0000 - 61.0000 years and the median was 40.0000 years. In group- without HIV, the mean of age (mean $\pm$ s.d.) of patients was 33.7533  $\pm$  4.8396 years with range 27.0000 - 45.0000 years and the median was 32.0000 years. Association of age in two groups was statistically significant (p<0.0001). In group-HIV, the mean of CD4 Count (mean $\pm$ s.d.) of patients was 583.3000  $\pm$  260.5644 cells/ microlitre with range 136.0000 - 1277.0000 cells/ microlitre and the median was 542.0000 cells/ microlitre. In group- without HIV, the mean of CD4 Count (mean $\pm$ s.d.) of patients was 1255.3067  $\pm$  127.4340 cells/ microlitre with range 1090.0000 - 1480.0000 cells/

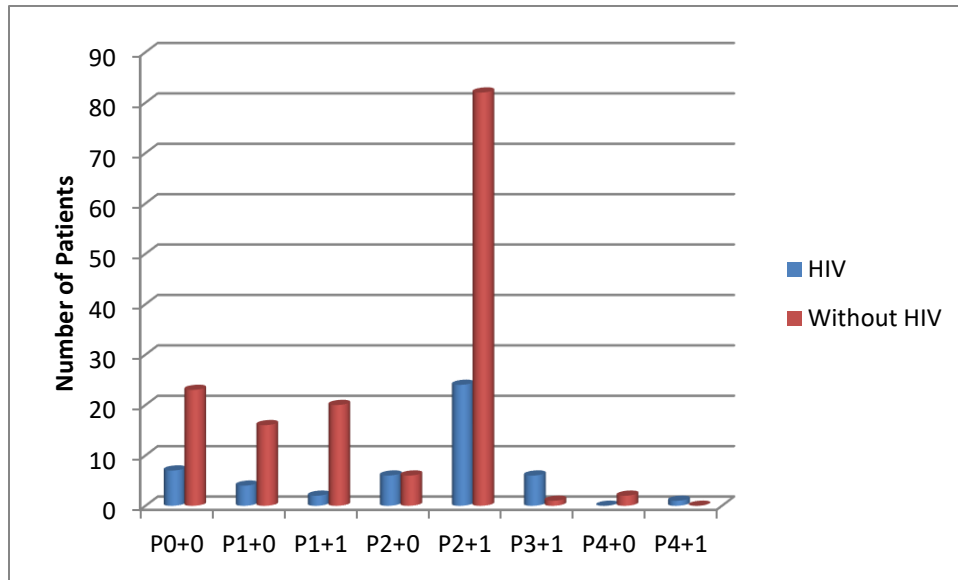
microlitre and the median was 1208.5000 cells/ microlitre. Association of CD4 Count in two groups was statistically significant ( $p < 0.0001$ ). (Table 1)

**Table 2. Distribution according to multiple sex, white discharge and report**

		<b>HIV</b>	<b>Without HIV</b>
<b>Multiple sex</b>	<b>No</b>	40	150
	<b>Yes</b>	10	0
<b>White discharge</b>	<b>No</b>	20	116
	<b>Yes</b>	30	34
<b>Report</b>	<b>ASCUS</b>	0	2
	<b>HSIL</b>	6	6
	<b>Inflammatory Smear, NILM</b>	16	0
	<b>LSIL</b>	11	15
	<b>NILM</b>	16	126
	<b>Squamous Cell CA</b>	0	1
	<b>Squamous Cell Carcinoma</b>	1	0

In group-HIV, 10(20.0%) patients had multiple sex. In group- without HIV, no patients had multiple sex. Association of multiple sex in two groups was statistically significant ( $p < 0.0001$ ). In group-HIV, 30(60.0%) patients had white discharge and in group- without HIV, 34(22.7%) had white discharge. Association of white discharge in two groups was statistically significant ( $p < 0.0001$ ). In group-HIV, 6(12.0%) patients had HSIL report, 16(32.0%) patients had Inflammatory Smear, NILM report, 11(22.0%) patients had LSIL report, 16(32.0%) patients had NILM and 1(2.0%) patients had Squamous Cell Carcinoma report. In group- without HIV, 2(1.3%) patients had ASCUS report, 6(4.0%) patients had HSIL report, 15(10.0%) patients had LSIL report, 126(84.0%) patients had NILM report and 1(0.7%) patients had Squamous Cell CA report. Association of report in two groups was statistically significant ( $p < 0.0001$ ). (Table 2)

Figure 1 : Distribution according to parity



Chi-square value: 25.0238; p-value: 0.0008

In group-HIV, 7(14.0%) patients had P0+0 parity, 4(8.0%) patients had P1+0 parity, 2(4.0%) patients had P1+1 parity, 6(12.0%) patients had P2+0 parity, 24(48.0%) patients had P2+1 parity, 6(12.0%) patients had P3+1 parity and 1(2.0%) patients had P4+1 parity. In group-without HIV, 23(53.0%) patients had P0+0 parity, 4(8.0%) patients had P1+0 parity, 20(13.3%) patients had P1+1 parity, 6(4.0%) patients had P2+1 parity, 1(0.7%) patients had P3+1 parity and 2(1.3%) patients had P4+0 parity. Association of parity in two groups was statistically significant (p=0.0008). (Figure 1)

### Discussion :

The incidence of HIV is on the rise.<sup>20</sup> As per HIV estimates 2008-09, there are an estimated 23.9 lakh people living with HIV / AIDS in India with an adult prevalence of 0.31 percent in 2009 (NACO report 2011).

HIV positive women have a high rate of persistent HPV infections, and a higher rate than HIV negative women with the types of HPV that are associated with the development of high grade dysplasia and cervical cancer.<sup>21</sup>

In present study in group-HIV, the mean of age (mean±s.d.) of patients was 41.5600 ± 7.9338 years with range 27.0000 - 61.0000 years and the median was 40.0000 years. In group- without

HIV, the mean of age (mean±s.d.) of patients was  $33.7533 \pm 4.8396$  years with range 27.0000 - 45.0000 years and the median was 32.0000 years. Association of age in two groups was statistically significant ( $p < 0.0001$ ). In group-HIV, the mean of CD4 Count (mean±s.d.) of patients was  $583.3000 \pm 260.5644$  cells/ microlitre with range 136.0000 - 1277.0000 cells/ microlitre and the median was 542.0000 cells/ microlitre. In group- without HIV, the mean of CD4 Count (mean±s.d.) of patients was  $1255.3067 \pm 127.4340$  cells/ microlitre with range 1090.0000 - 1480.0000 cells/ microlitre and the median was 1208.5000 cells/ microlitre. Association of CD4 Count in two groups was statistically significant ( $p < 0.0001$ ) which were obtained compared to Amphan et al 28.87%, Leibenson et al 39.75%, Jennifer et al 92.67% and BM Jha et al 10.46% cases.<sup>22</sup>

This result is comparable with recent study of Seethalakshmi et al (58.8% versus 43.75%) and that of O'Sullivan MJ et al.<sup>23</sup>

studies from Europe, the United States and Africa have provided figures that can be compared, and show highly concordant results: prevalence rates of abnormal PAP smear in these studies ranged from 10% to 24% among HIV positive women, from 4 to 10% amongst HIV negative women.<sup>24</sup>

In present study in group-HIV, 10(20.0%) patients had multiple sex. In group- without HIV, no patients had multiple sex. Association of multiple sex in two groups was statistically significant ( $p < 0.0001$ ). In group-HIV, 30(60.0%) patients had white discharge and in group- without HIV, 34(22.7%) had white discharge. Association of white discharge in two groups was statistically significant ( $p < 0.0001$ ). In group-HIV, 6(12.0%) patients had HSIL report, 16(32.0%) patients had Inflammatory Smear, NILM report, 11(22.0%) patients had LSIL report, 16(32.0%) patients had NILM and 1(2.0%) patients had Squamous Cell Carcinoma report. In group- without HIV, 2(1.3%) patients had ASCUS report, 6(4.0%) patients had HSIL report, 15(10.0%) patients had LSIL report, 126(84.0%) patients had NILM report and 1(0.7%) patients had Squamous Cell CA report. Association of report in two groups was statistically significant ( $p < 0.0001$ ).

Other risk factors associated with HIV was considered in the study viz; number of partners, addictions, method of contraception etc, significant association was found.<sup>25</sup>

P value in present study was  $<0.05$ . It signifies that the difference is statistically significant and signifies that HIV infected women have more risk of having cervical epithelial abnormalities as compared to general population.

In the present study association of parity in two groups was statistically significant ( $p=0.0008$ ). Study conducted by Kusumam VN found that PAP smear abnormality (overall), prevalence of LSIL was significantly higher among HIV positive patients. It was not associated with parity.<sup>26</sup>

### **Conclusions**

With the dual epidemic of HIV and cervical cancer gaining a foot hold and reaching pandemic proportions, our present study of estimating the prevalence of Pap Smear abnormalities in HIV seropositive women and the risk factors associated with them has shown and the risk factors associated with them has shown a twofold increased risk of cervical cytological abnormalities, while the risk factors for abnormal cervical smears like younger age, lower CD4counts, longer duration of disease, and ART/HAART therapy were found to be of no statistical significance.

This study represents a relationship between HIV seropositive and abnormal Pap smears and stresses the need for periodic Pap smear screening in this high-risk population to decrease the global burden of cervical cancer.

Worth noting is an underestimate of the true prevalence of precancerous lesions as we have studied with conventional Pap smear screening wherein the superiority of liquid based cytology has been proved

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**Conflict of interest:** None declared

**Ethical approval:** The study was approved by the institutional ethics committee



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