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RESEARCH ARTICLE

A study on modifiable and nonmodifiable risk factors associated with developing pap smear abnormalities in HIV-positive women

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ABSTRACT

Objectives: This study aims to estimate the frequency of abnormal pap smears with early detection of lesions, to assess the risk factors in HIV-positive women and the process increase their awareness. **Methods:** Hospital-based cross-sectional analytical study carried out in Gauhati Medical College and Hospital from March 2019 to June 2020. Pap smears from 100 HIV-positive women (18 - 60 years) were taken randomly. The association between risk factors and pap smear abnormality was determined by calculating chi-square, p-value, attributable risk and relative risk. **Results:** Most cases had NILM (79%). 19% intraepithelial lesions (14 ASCUS, 3 LSIL, 2 HSIL) and zero invasive cancer. Lower CD4 count (<200cells/cu mm), unhealthy cervix, and postmenopausal state are significant unmodifiable risk factors while early age at coitarche (<18 years), multiple partners of spouse, duration of HAART are significant modifiable risk factors for developing pap smear abnormality (P <0.05). Women with multiple sexual partners were found to have a higher relative risk of developing ECA. **Conclusion:** HIV-positive women have various risk factors (modifiable as well as non-modifiable) which have a significant association with developing cervical epithelial abnormalities which if not intervened will lead to invasive lesions in the cervix. It is pertinent to educate these women about the modifiable risk factors which can play a great role in risk reduction and prevention of cervical cancer.

Keywords: Cervical cancer, HIV positive, epithelial cell abnormality (ECA), CD4 count, risk factors, HAART.

Cervical cancer is one of the leading causes of cancer mortality amongst women amounting to around 500,000 cases annually and 275,000 deaths per year, accounting for around 7.5% of all female cancer deaths.[1] Majority of these deaths occur in third world countries which accounts for around nine in ten (87%) cervical cancer deaths occurring in less developed regions (WHO, 2008, IARC)[2]. It is one of the leading causes of disability-adjusted life years. On the brighter aspect, it is one of the preventable cancers due to its slow progression, long latent time with cytology detectable precancerous lesions and availability of effective treatment options. Human papillomavirus infection is the major etiological agent identified in the pathogenesis of cervical cancer. HPV types 6 and 11 are categorized as low-risk strains and types 16,18,31,33,35,45,51, 52, and 58 are frequently found in invasive cancers and are implicated as main factors in carcinogenesis [3]. HPV infection clears in 6-24 months spontaneously. However, women with HIV-positive status have a higher risk of HPV infection and its persistence as well as infection by multiple HPV genotypes due to their immunocompromised state. They have a ten times higher risk of progression to invasive cancer (Palefsky et al) [4]. It has also been reported that the persistence of HPV infection is consistent with decreasing CD4 count and

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increasing HIV RNA levels. These correlations prompted CDC to include invasive cervical cancer in the AIDS surveillance case definition in 1993. Even though the AIDS-related malignancies are greatly attributed to immunodeficiency but the relationship between cervical neoplasia and HIV is inclusive of the common sexual behavioural risk factor as well as the interaction between the HIV-infected host cell and HPV at a molecular level.

The incidence of cervical cancer and related mortality and morbidity has markedly reduced in most developed countries due to the effective implementation of screening programmes. An overwhelming majority of women in India are deprived of standard screening programmes due to resource limitations. In the same setting, the global HIV pandemic has hit India and has had an enormous impact on women, particularly of reproductive age group. Most of them are incidentally diagnosed during antenatal checkups and are not in regular follow-up for screening tests and antiretroviral therapy owing to ignorance, illiteracy, poverty and fear of social ostracization. With the intervention of antiretroviral therapy, there has been a considerable increase in the life expectancy of HIV-positive people. It has also brought along the threat of cancer development in this population by exceeding the latent period of conversion of precancerous lesions. Enhancing early detection and treatment of precancerous lesions through screening could reduce the burden of cervical cancer in this section.

A Pap smear is a simple, cheap noninvasive tool for the early detection of cervical precancerous lesions. Pap smears can be collected by grassroots-level workers as it is an easy procedure and doesn't require much skill or training provided there is the availability of a trained and experienced cytopathologist for interpretation of the smears. It has high sensitivity with low false positives. This study aims to determine the prevalence and patterns of abnormal cytology and their correlation with risk factors like parity, polygamy, CD4 count, HAART etc amongst HIV-positive women attending Gauhati Medical College and Hospital.

Aims and objectives

- To estimate the frequency of abnormal pap smears and detects precancerous, cancerous and inflammatory lesions in HIV-positive women.
- To assess whether HIV-positive women have an increased likelihood of developing dysplastic and neoplastic changes
- Increase awareness about the risk factors and value of pap smear as an integral part of preventive care for HIV

Materials and methods

This hospital-based cross-sectional analytical study was carried out in the department of obstetrics and gynaecology, Gauhati Medical College and Hospital during the period from March 2019 to June 2020. Pap smears from one hundred HIV-positive women between the age group 18 years to 60 years attending antenatal OPD, gynaecology OPD and ART centre were taken randomly who fulfilled the inclusion criteria after taking written and informed consent. Strict confidentiality of the patients' identities was maintained throughout the study.

Inclusion criteria -

All HIV-positive women attending antenatal OPD, gynaecology OPD, ART centre, GMCH from March 2019 to June 2020

Exclusion criteria -

1. Unwilling patients
2. HIV-positive females less than 18 years and more than 65 years
3. HIV-positive women who have undergone total hysterectomy
4. HIV-positive women diagnosed with carcinoma cervix

Steps -

1. Written and informed consent was taken in a specified consent form.
2. Filling up the proforma
3. History
4. General examination
5. Local inspection of the perineum
6. Speculum examination of cervix and vagina
7. Pap smear collection: The patient was placed in a lithotomy position after she had evacuated her bladder. An appropriate-sized plastic disposable Cusco's speculum was gently inserted into the vagina. The cervix was fully visualised and the squamocolumnar junction was identified. The Ayre's spatula was inserted in the vagina and rotated 360 degrees. The scraping was then evenly spread on a labelled glass slide and then immediately fixed using 95% isopropyl alcohol in a Koplin's jar. An endocervical sample was then taken using the cytobrush and a smear is prepared and fixed like the previous slide. The slides were

then stained and analysed according to Bethesda 2001 reporting guidelines.

8. Recording the findings
9. Slides were sent to the Pathology Department, Gauhati Medical College and Hospital for reporting
10. CD4 counts of all the patients were obtained.

The association between risk factors and pap smear abnormality was determined by calculating the chi-square value and p-value by Fisher's exact test. Attributable risk and relative risk of the study population in relation to risk factors were also determined by Koopman asymptomatic score and a comparison was drawn between the association of modifiable and non-modifiable risk factors.

Results

The mean age of the study population was found to be 35.69 years with a standard deviation of 9.74 years (standard error of the mean: 0.97). The mean age of coitarche was 19.84 +/- 3.25 years (standard error of the mean: 0.32). 88% of women were in the reproductive age group (menstruating). 14% of the studied subjects were pregnant. The mean parity was found to be 2.23 with a standard deviation of 0.6. *Candida vaginalis* was the most commonly identified organism (66.6%) followed by *Trichomonas vaginalis* 33.3%. 50% of the cases with genital tract infection had CD4 count between 200-500 cell/cu mm. Both the patients with *Trichomonas vaginalis* infection had CD4 count between 200-500 cells/cu mm.

Table 1: Pap smear results

Results	No of cases	%
NILM (negative for intraepithelial lesion or malignancy)	79	79%
(a) No inflammation	28	28%
(b) Inflammation	45	45%
(c) Organisms	2	2%
(d) Inflammation+Organism	4	4%
ASCUS	14	14%
LSIL	3	3%
HSIL	2	2%
Invasive cervical cancer	0	0%
Inadequate smear	2	2%

ASCUS - Atypical squamous cells of undetermined significance, LSIL - Low-grade squamous intraepithelial lesion, HSIL - High-grade squamous intraepithelial lesion.

Out of the 100 Women, 89% had never undergone any previous pap smear tests. 5% were not aware of pap smear tests at all and of the 6% women only half of them knew about their pap smear results (table 1). Various established risk factors for cervical carcinoma were studied and their association with the development of pap smear abnormality in the study group was estimated (table 2).

The maximum number of cases were asymptomatic (68%), 16% had complaints of discharge per vaginum, 2% had a history of post-coital bleed, 4% had irregular bleeding per vaginum, 9% had pain abdomen and 1 patient had pruritus vulva which was diagnosed as leukoplakia on examination and biopsy.

26 women had unhealthy cervix on per-speculum examination of which 46.1% showed normal pap findings and 50% showed abnormal pap results. 90.5% of women with healthy cervix on per-speculum examination had normal pap smear cytology and only 8.1% of these women had abnormal pap smear results.

In this study, we have found that CD4 count below 200cells/cu mm, unhealthy cervix on per-speculum examination, early age at coitarche (<18 years), multiple partners of husband and postmenopausal state are significant risk factors for developing pap smear abnormality ($p<0.05$). However, the association of multiple sexual partners and spouse with multiple partners to that pap smear abnormality may not bear the real picture as many women might not have revealed their true history. In our study, even though the association of multiple sexual partners was not found to be significant, such women were found to have a higher relative risk of developing pap smear abnormality.

Table 2: Types of risk factors

Modifiable risk factors	P Value	Non-modifiable risk factors	P value
HAART duration	<0.001	Age	0.084
Multiple partners	0.08	Unhealthy cervix	<0.0001
Multiple partners of spouse	0.032	Menopause	<0.0001
Age at coitarche	0.0013	CD4 count	<0.0001
Pregnancy	0.29	Gynaecological complaints	0.09
Parity	0.23		

HAART intake was not found to significantly reduce the odds of pap smear abnormality. However, patients with HAART intake for more than 5 years had a relative risk of 0.54 as compared to their counterparts which indicate the effect of risk reduction by >5 years of HAART intake. Parity, complaints of vaginal discharge, bleeding per vaginum, post-coital bleed, age, pregnancy, and multiple partners were not found to be significantly associated with pap smear abnormality ($p> 0.05$). Most of the patients who had CD4 count <200cells/cu mm showed genital tract infections. *Candida vaginalis* (4 cases) was the most common infection amongst the two organisms identified. 2 cases showed infection with *Trichomonas vaginalis.*

Discussion

Different studies have shown the different distribution of epithelial abnormalities. Our study matches with the findings of Wahane et al[5] and Darvishi et al[6] in terms of the prevalence of ASCUS. In regards to HSIL it is quite

Table 3: Comparison of relative risk, attributable risk and chi-square value against the tabulated value of the risk factors.

Modifiable risk factors	RR	AR	Chi sq value		Non-modifiable risk factors	RR	AR	Chi sq value	
			Calculated value	Tabulated value				Calculated value	Tabulated value
HAART duration	0.54 (CI 0.17 to 4.78)	0.1 (CI 0.04 to 0.31)	374.64	43.773 at 30 df in 5% probability level	Age	0.71(CI 0.05-3.47)	0.4 (CI 0.03 to 5.4)	3.18	12.592 in 5% probability level
Multiple partners	2.85 (CI 0.99-5.94)	0.32 (CI -0.004 - 0.69)	10.45	12.592 at 6 df in 5% probability	Un-healthy cervix	6.32 (CI 2.76 to 14.60)	0.4 (CI 0.2 to 0.6)	121.18	28.8 at18 df in 5% probability level
Multiple partners of spouse	1.81 (CI 0.81-3.89)	0.12 (CI -0.05 to 0.34)	17.54	12.33 at 10 df in 5% probability	Menopause	5.93 (CI-2.56 to 10.22)	0.54 (CI 0.21-0.76)	32.3	14.067 at 7 df in 5% probability
Age at coitarche	3.80 (CI 1.7 to 8.2)	0.32 (CI 0.10 – 0.52)	24.51	21.026 at 12 df in 5% probability	CD4 count	7.43 (CI 4.13 to 13)	0.74 (CI 0.5 to 0.86).	51.28	21.026 at 12 df in 5% probability
Pregnancy	0,34 (CI 0.05-1.62)	0.13 (CI 0.013-0.43)	7.51	14.76 in 10df at 5 % probability	Gynaecologic-al complain	0.79 (CI 0.56 to 1.03)	0.12 (CI -0.04 to 0.33).	7.28	21.45 at 6 df in 5% probability
Parity	0.406 (CI 0.107-1.36)	0.13 (CI -0.002 to 0.36)	12.87	34.92 at 10 df in 5% probability					

comparable to Wahane et al, Vani YS et al [7], Gichuhi et al [8] and Apeksha Madan et al [9]. Getinet et al[10], Darvishi et al and Ugboaja et al [11] have found a higher prevalence of HSIL. For LSIL, our study is comparable to the findings of Apeksha Madan et al, Darvishi et al, Gosai et al, Getinet et al and Wahane et al. Vani YS et al, Vafae et al [12], Ugboaja et al and Gichuhi et al have found a higher prevalence of LSIL. The prevalence of invasive cervical cancer is almost similar in all the studies including the present study. The variations in the observations of different studies may be attributed to the differences in the respective sizes of the study populations.

No statistical significance was noted between multiple sexual partners and abnormal pap smear results in the studies conducted by Kusuman VN et al[13], Gichuhi et al, Vafae et al and Bawa et al, Ugboaja et al. The study by Getinet et al found that more than 2 sexual partners are significantly associated. These women had a high risk for developing ECA when compared to their counterparts with one or two sexual partners (AOR 3.2, 95 % CI: 1.0−10, p=0.048). Sansone et al[14] have found that five or more lifetime sexual partners increased the risk of developing cervical epithelial abnormalities (OR: 3.47; 95% CI: 2.55–10.69; P < 0.001). In our present study, multiple partners have a p-value of 0.08 in relation to the development of epithelial abnormalities however, the relative risk is significant. Such a finding may be confounded by the fact that all subjects might not have truly disclosed their sexual practices and promiscuity because most of them are either married or widowed, bound by socio-cultural constraints. A study with a larger study population will perhaps give a more reliable report in this aspect. However, we found a significant association between multiple partners of spouse and of abnormal pap smear findings in our subjects.

Vafaei et al, Vani YS et al, Gichuhi et al, and Getinet et al reported that CD4 count <200 cells/cu mm had a significant association with pap smear abnormality. Jaya Chakravarty et al [15] CD4 count of ≤ 350 cells / cu mm was a risk factor with an odds ratio of 2.464. Apeksha Madan et al have found a borderline significant association between CD4 count and abnormal pap smear findings. Ugboaja et al reported that CD4 count <300 cells/cu mm is a strong predictor of developing cervical abnormalities with OR 0.037, p=0.00. The present study has also found a highly significant correlation with low CD4 count.

The present study did not find any significant association between administration of HAART with pap smear abnormality, p-value calculated was 0.55. However, 5 years of HAART intake had shown a relative risk of 0.54 (95% CI 0.17 to 4.78) and an attributable risk of 0.1 thus implying its highly significant risk reduction effect. This study has comparable findings of abnormal cervical cytology amongst the population on ART to that of the studies conducted by Apeksha Madan et al, Gichuhi et al, Vani YS et al, Jaya Chakravarty et al and Ugboaja et al. However, Kusuman et al have found a higher prevalence (45%) of cervical abnormalities amongst the group on ART. In the group not on ART, our study is only slightly comparable to Kusuman et al and Jaya Chakravarty et al.

These variations are probably due to the iceberg phenomenon of HIV cases that are long untraced due to a

lack of healthcare reach and utilization by the population. Many patients have been diagnosed at quite an advanced stage of HIV infection and these patients are likely to have comorbidities associated with immunosuppression including abnormal cervical cytology as in our study, most of the cases who were not on ART were newly diagnosed cases of HIV.

Kavitha et al [16] found that 70.6% of cases with abnormal cervices had positive pap smear findings whereas only 18.4% of women with normal cervix had abnormal pap results. Vafae et al reported that 26% of HIV patients had lower genital tract infection (10%),gardenella9% trichomonas, 7% candida vaginalis) and 26% had abnormal pap smear results. 7% of patients had complaints of post-coital bleeding. Pundhir et al[17] 2009 reported a higher incidence of sexually transmitted infections manifesting as cervical erosion and vaginal discharge. 66% of women with unhealthy cervix had SIL on cytology. In our study, we did not find a significant association between pap smear abnormality and presenting complaints of vaginal discharge, irregular bleeding per vaginum, post-coital bleed and pruritus vagina. However, one patient presenting with pruritus vagina had leukoplakia and VIN I was detected on biopsy. An unhealthy cervix on per-speculum examination had a significant association with cervical epithelial abnormality.

Ugboaja et al reported early coitarche increased the risk of developing pap smear abnormality. Getinet et al found early age at first sexual contact (<15 years) was a significant risk factor for the development of ECA (AOR 5.2, 95 % CI: 1.5− 17.9, p=0.009). Bawa et al[18], Vafaei et al reported no significant association between age at coitarche and pap smear abnormality. In our study, we found that lower age at coitarche (<18 years) is significantly associated with abnormal pap smear results,

Getinet et al reported that women with high parity (>4) were ten folds more likely to develop ECA (AOR10.9, 95 % CI; 4.2−16.8, p<0.001) than women with parity lower than three. Aparna Wahane et al observed that cervical epithelial cell abnormalities were found more commonly in women with a parity of more than two (21.1%). Vafae et al, Bawa et al, and Ugboaja et al reported no significant association of parity with cervical cytology. We have found that higher parity doesn't have an effect on developing pap smear abnormality. P value for ≥ 4 previous pregnancies is 0.23.

Conclusion

From the results obtained from this study, it is evident that HIV-positive women have various risk factors (modifiable as well as non-modifiable) which have a significant association with developing cervical epithelial abnormalities which if not treated and followed up will lead to invasive lesions in the cervix. Modifiable risk factors are age of coitarche, multiple partners of spouse and duration of HAART intake. Women with multiple partners have a high relative risk of pap smear abnormality which points towards its positive association. Whereas nonmodifiable significant risk factors are post-menopausal state, low CD4 count and unhealthy cervix on per-speculum examination. It is very pertinent to educate these less fortunate women about the modifiable risk factors which can play a great role in risk reduction and prevention of cervical cancer in HIV-positive women by behavioural modifications such as the adoption of protected and monogamous sexual relationships, discouraging early coitarche ie before 18 years. Many gynaecological complaints like pruritus, leucorrhoea etc can also be reduced by adopting practices of safe sexual intercourse and basic hygiene. A study using a larger number of women will give a more reliable report with respect to the association of various risk factors and premalignant lesions of the cervix among HIV-infected women in our environment.

Conflict of interest: None. **Disclaimer:** Nil.

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