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ASSOCIATION OF ABNORMAL CERVICAL CYTOLOGY WITH HIV POSITIVITY AMONG WOMEN OF REPRODUCTIVE AGE GROUP

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INTRODUCTION

HIV infection causes impaired cell mediated immunity which in turn is responsible for persistent HPV infection & increase risk for abnormal PAP Test.[1,2] HIV seropositivite women if affected by high risk HPV types it progresses rapidly to precancerous lesion and then cervical cancer as compared to seronegative women. Frequent screening and elimination of precancerous lesion under cervical cancer prevention program is the only way of blocking the oncogenesis. There are 110 types of HPV infections known and out of which only half infect the genital tract. According to IARC WHO classification only 13 types are carcinogenic and rest 7 are possibly carcinogenic. Out of carcinogenic ones are HPV -16,18,31,33,35,39,45, 51, 52, 56,58, 59 and 68.[3] Immunosuppresed state of HIV prevents host from effectively eliminating HPV from the target tissues and make it prone for CIN and

precursor lesion. The relationship among human immunodeficiency virus, Human pappiloma virus, and development of Cervical Intraepithelial Neoplasia are undoubtedly complex and incompletely understood. Present study is an attempt to find out the prevalence and relationship of abnormal cervical cytology and HPV infection in HIV positive women.

AIMS AND OBJECTIVES

This study was conducted to determine the gynaecologic characteristics related with HIV infection, and also to find out prevalence of abnormal cytology either due to Human Papillomavirus, and highlighting the importance of cervical cancer screening program in HIV positive women.

MATERIAL AND METHODS

This was a cross-sectional, case control study which was conducted in the Department of Obstetrics & Gynaecology and Antiretroviral Treatment (ART) clinic of a tertiary care centre of North India after ethical clearance from the Institute. Study was conducted on total 190 women. In the study group **(Group A)** 95 HIV positive women were included from Art clinic and equal numbers of HIV negative women were recruited as controls **(Group B).** Controls were recruited from women of almost same age and parity coming to gynaecology OPD for any gynaecological problem or advice.

Inclusion Criteria

1. All consenting HIV positive women during the study period of one year.

Exclusion Criteria

1. Subjects not willing to participate in the study.
2. Previously diagnosed abnormal cervical cytology.

All subjects were counselled and an informed consent was taken. HIV status of control group was confirmed by blood test (ELISA). All HIV positive women of study group were also assessed for CD4 cells counts. They were evaluated thoroughly by history and examination. Specimen was collected from the cervix using cytobrush for HPV DNA testing for cytology. Results of cytology were classified by using the Bethesda classification system. After collection specimen was placed in a transport medium (Phosphate buffer Saline Medium) and frozen at -20C until testing. HPV DNA was detected with the polymerase chain reaction (PCR) based method using HPV L1 consensus primer (MY11 and MY09) and control primer

set PC04/GH2O. Positive sample was defined as those containing an ethidium-bromide-stained band of the correct mol. wt.(450bps) after amplification and 1.5% agrose gel electrophoresis. Furthermore, adequacy of negative sample for PCR analysis, i.e absence of inhibitors, was assessed by the amplification of a fragment of β-globin gene.For HPV DNA-positive sample, and typing was done by PCR using type specific primers for HPV16, HPV18, HPV6 and HPV11.

Data analysis

Data analysis was done using the relevant statistical tool. The statistical significance was determined by using Chi square test with 5 percent level of significance (P<0.5), and Power of the study was 90%. Women were compared using student t test for continuous variable and Chi – Square for categorical variables factor like age, CD4 count, HIV -1 RNA, education employment, parity, complaints. number of sexual partners and age of first intercourse.

RESULTS

While comparing the Demographic characteristics of cases and controls, no significant difference was found in terms of age, parity, socioeconomic status, educational status etc. Most of the subjects of both groups belonged to age group 25-34 years. Mean age of the cases was 31.9 years. Most of the women in both groups were multiparous. Comparing the contraceptive use condom was the most used contraceptive in HIV positive women [16%] and Intrauterine contraceptive device was the most used contraceptive in HIV negative women [7.4%].

Table 1: Distribution of clinical symptoms in group A and group B.

Clinical symptoms	HIV Positive(Group A)		HIV Negative(Group B)	
	No	%	No.	%
Discharge	17.0	17.9	15.0	15.8
Dysuria	6.0	6.3	3.0	3.2
Infertility	0.0	0.0	3.0	3.2
Itchiness of vulva	4.0	4.2	0.0	0.0
Menorrhagia	0.0	0.0	9.0	8.4
Pain in lower abdomen	7.0	7.4	16.0	16.8
Post coital bleeding	0.0	0.0	1.0	1.1
Prolapse	1.0	1.1	1.0	1.1

P =0.006

Majority of women had no symptoms, percentage of such women was 63.2% in group A and 49.5% in group B.Discharge per vaginum was the most common symptom in both the groups.

Mucoid discharge was most common presentation [27.36% women in group A and 21.1% in group B] and curdy white discharge was least present[7.36% and 0% respectively].

Table 2: Distribution of abnormal cytology among both groups.

Cytology	HIV positive (group A)		HIV Negative (group B)	
	No(79)	%	No(51)	%
Inflammation	62	78	46	90
HPV infection	8	10	5	10
AGC	2	3	0	0
ASCUS	1	1	0	0
LSIL	4	5	0	0
HSIL	1	1	0	0
Cell suspicious of malignancy	1	1	0	0

Out of 190 subjects 130 had abnormal cytology. In Group A 83% had abnormal cytology and in Group B 48.45% had abnormal cytology and rest were normal. Out of 130 abnormal smear majority of them had inflammatory smear (65% and 48.4% in group A and group B respectively).In inflammatory smear there were women had inflammation with Trichomonas Vaginalis, inflammation with follicular cervicitis and candida infection one of each above found only in group A.HPV infection on cytology smear found in eight women in group A and five women in group B.Out of eight women one had HPV infection associated with Gardenella organism in group A. Premalignant condition like AGC, ASCUS, LSIL, HSIL and cell suspicious of malignancy were found in only group A (3%,1%,5%,1%,1% respectively).

Table 3: Distribution of HPV among study and control group.

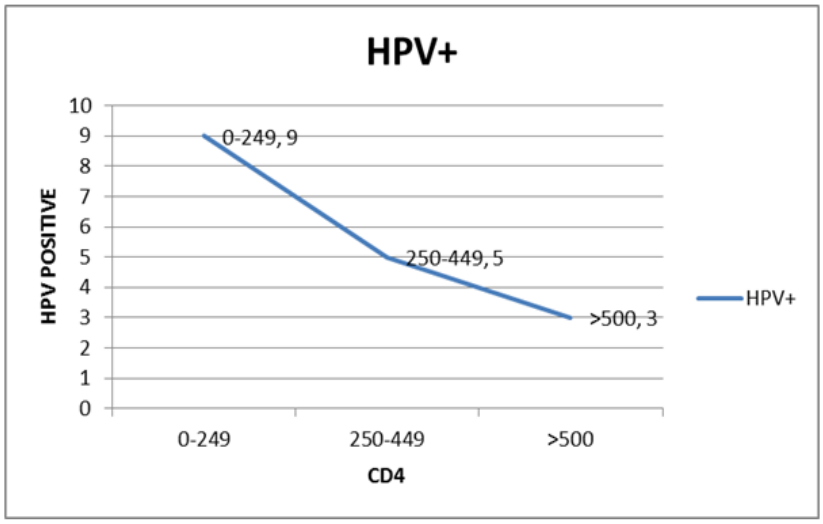
HPV	HIV positive(group A)		HIV Negative(group B)	
	No(95)	%	No(95)	%
Negative	78	82.3	88	92.6
Positive	17	18.6	7	7.4
HPV 16 type	16	17.6	7	7.4
HPV 16+18 type	1	1	0	0

P=0.043

There was significant difference in association of HPV DNA between study and control group (p= 0.043). The percentage of HPV DNA positivity among study group was 18.6% and in control group it was only 7.4%. Out of positive HPV groups, HPV 16 was more prevalent in both groups (17.6% v/s 7.4%). Combined HPV 16 +18 was seen only in one patient in group A.

Out of 95 HIV positive women 27.37% had CD4 count below 249, 44.21% women had CD4 count between 250-499 and 28.42% women had CD4 count >500.

Table 4: CD4 count and HPV.



Pearson correlation (-0.982) CD4 count <249 HPV DNA positivity were 53% and when increased number of CD4 counts in HIV positive women decrease the percentage of HPV DNA positivity (CD4 counts between 250-499, percentage of HPV DNA positivity were 5 and CD4 count >500 HPV DNA positivity was 19%).

There was no significant association found between ART treatment and HPV DNA positivity. Out of 17 HPV positive, 2 had not started ART treatment and 15 were on ART treatment. HPV positivity was more (88%) in women in taking ART VS 12% in women not taking the same. Out of 95 HIV positive twenty seven women were not on any ART treatment. Women who were taking ART for 6-12 months duration were 15.8%, for 12.1-24 months duration were 15.8% and for more than 24 months were 28.4%.

DISCUSSION

Acquisition of both Human pappiloma Virus and Human immunodeficiency virus is related to common behavioural risk factors such as multiple sex partners which make the assessment of casual relationship difficult to assess. The estimated time interval between initial HPV infection and development of cervical cancer is 20 years and dysplasia occurs in stages. Persistent HPV infection may lead to CIN 1 which usually resolve on its own in majority of cases. Only in minority of cases these lesions progress to CIN 2, CIN 3, adenocarcinoma in situ and cervical cancer. If HPV infection occurs in HIV positive women there is much more

rapid progression to higher grade and invasive lesions. In fact chances of detection of HPV infection increases rapidly within early years of seroconversion which is suggestive of mucosal immune dysfunction or HIV related change in the molecular pathway leading to cervical cancer.[4] Immunosuppression makes the infected women more prone for persistent infection and progressive dysfunction in immune system along with failure to curb the neoplastic changes. In present study HPV infection was significantly higher in study group than control group [18.6% Vs 7.4%]. This observation can be explained by the fact that immunosuppression due to HIV infection causes impaired clearance of HPV thereby causing positivity for the same more in the study group.[5,6] Another study has reported co- infection with HPV infection was seen in 63.2% in cases as compared with 30.4% in HIV seronegative women.[7] High risk HPV type like HPV 18 were exclusively seen in HIV positive women and HPV 16 was significantly higher in study group [17%vs 7.4%]. Another author reported chances of Abnormal PAP smear to be 4.3 fold higher in HIV positive women.[8] In present study abnormal cytology in HIV seropositive women was found to be 83.2% which was significantly higher than HIV negative women where it was 48.45% [p=0.015]. Cytological features like AGC, ASCUS, LSIL and HSIL were exclusively seen in the study group. Cell suspicious of malignancy was found in 1% of the study group while none of the control group. In another study L. Stewart et al reported prevalence of abnormal cervical cytology as high as 38.3% among HIV infected women as compared to 16.2% in uninfected women. This study also revealed chances of detection of HPV infection is 9% Vs 13% in benign smears, 12% Vs 23% in ASCUS, 14% Vs 32% in LSIL, 25% Vs 41 % in HSIL or malignant smears.[7] In present study we also found the prevalence of HPV DNA significantly higher in HIV positive women than in controls [18.6% Vs 7.4 %]. Although the difference is much higher in other studies in comparison to present study can be explained due to variation in sample size. Although there are some studies which after years of observation have reported that most abnormal Papanicolaou tests in HIV –Seropositive women are atypical or low grade lesions not the high grade lesions leading to the high grade lesions leading to precancerous state.[9,10] HPV 16,18 and other high HPV Types are the risk factors of cervical cancer and for cervical intraepithelial neoplasia and its precursors.[7,9,10,11,] A significant correlation was found between CD4 count and HPV DNA reports with p value 0.121& Pearson correlation coefficient - 0.982. 53% of HPV DNA positive women had CD4 count < 250 / mm^3, 31% had CD4 count 250-499/ mm^3. Many other studies have shown the similar observation.[12,13] In present study no significant association was found between duration of ART and HPV positivity. it was observed that the HPV DNA positivity was more i.e. 88% in women taking

HAART versus 12% in women not taking the same. This could be because the women recruited for HAART were the ones with low CD4 count in which the lower immunity caused higher prevalence of HPV infection. Due to lack of follow up in our study a definite conclusion regarding the HPV DNA positivity over a time period and regression of SIL in women taking HAART could not be made. Various studies have shown a contrary picture where the prevalence of HPV was lower in women taking HAART. Linda Ahdieh-Grant etal demonstrated that out of 312 women, 141 had lesions that regressed to normal cytology, with a mean time to regression of 2.7 years after HAART was introduced.[14] However in a study conducted by Paula Schuman et al it was found that SIL regression was less likely among HIV positive women with higher viral load and that HAART had no beneficial effect.[15] Observation is supported by a recent systemic review from Sub Saharan Countries.[6] Another aspect of this observation is that initiation of ART increases longevity. Moreover frequent contact with health facility increases chances of screening and diagnosis of cervical lesions,

CONCLUSION

It is inferred that the prevalence of abnormal cytology and HPV DNA positivity is higher amongst HIV positive women. Also, there is an association between HPV DNA positivity and CD4 counts which showed higher prevalence of HPV infection in subjects with low CD4 counts.

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