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■ Original Research Article

Cervical Cytology Pattern Among HIV Positive Women on HAART And HIV Negative Women in North Central Nigeria: A Comparative Study

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Abstract

Background: Abnormal cervical cytological pattern has been shown to be higher among Human Immunodeficiency Virus (HIV) positive women compared to their HIV negative counterparts. **Objective**: To compare the cervical cytology pattern among HIV negative and HIV positive women on Highly Active Antiretroviral Therapy (HAART). **Materials and Methods:** We conducted a prospective comparative study involving 157 HIV positive women on HAART for at least 6 months and 157 HIV negative women attending the medical outpatient department, Gynaecology, and family planning clinic at the Asokoro district hospital between 1st of March 2020 to 31st of October 2020. The clinical and socio-demographic data were gotten from the structured questionnaires administered and participant's case note. Blood samples were collected from each participant in the HIV positive group into the EDTA bottles and sent to the laboratory within 6 hours of collection for viral load analysis using polymerase chain reaction (PCR) and CD4 count analysis was done by flow cytometry using Cyflow. Counter II. The HIV testing for the HIV negative group was done using Determine HIV-1/2. Using the Ayre's spatula, cervical smear (Pap smear) was collected from each of the study groups, processed and read by consultant histopathologist. The data was analysed using SPSS version 21. Chi Square test was used for categorical variables to analyse, and the level of significance was set at $p < 0.05$. **Results**: Abnormal Pap smear results was higher in HIV positive group (n=3; 1.9%) compared to the HIV negative group (n=2; 1.3%) (p=0.88) but there was no statistical difference. Among those with abnormal Pap smear results , 1 (0.64%) had low-grade squamous intraepithelial lesion (LSIL), 1 (0.64%) had atypical squamous cells of unknown significance (ASCUS), 1 (0.64%) also had atypical glandular cells of unknown significance (AGUS) and none had high-grade squamous intraepithelial lesion (HSIL) in HIV positive group while 1 (0.64%) had LSIL and 1 (0.64%) also had ASCUS but none had neither AGUS nor HSIL in HIV negative group. Among HIV positive group, 1 (0.6%) had CD4 count of <200 cells/ml and 156 (99.4%) had CD4 count of >200 cells/ml. Also, 25 out of the 157 (15.9%) participants had viral load greater than 20copies/mm^3 while 132 (84.1%) participants had viral load ≤20copies/mm^3in the HIV positive group. **Conclusions**: The study showed that cervical cytology is not significantly different between HIV negative and positive women on HAART hence 3 yearly Pap smear screening similar to HIV negative women seems to be safe in low-risk women living with HIV on HAART.

Keywords: Cervical cytology, HAART, HIV negative, HIV positive

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INTRODUCTION

Cervical cancer is a preventable disease yet a very common malignancy worldwide and the second commonest cause of cancer related death in the developed world.[1] Annually, about half a million new cases are diagnosed worldwide, 79,000 in Africa constituting 25.4% of cancers in women on the continent.[2] About 14,943 women are diagnosed annually in Nigeria and 10,403 die from the disease.[3]

Cervical dysplasia is the precursor to cervical cancer and almost all cases of cervical dysplasia and cancer arise from infection by persistent oncogenic human papilloma virus (HPV).[4-6] The most common types responsible for most of the cervical cancer are HPV 16 and 18. HPV is a common sexually transmitted infection that usually clears within 8 to 24 months of the exposure and just a small percentage will develop cervical dysplasia.[7] The global HPV prevalence is 11.7% with West Africa having the 4th highest with a prevalence of 19.6%.[8]

Invasive cervical cancer is preceded by about 10 to 15 years of precursor lesions of the cervical intraepithelial neoplasia (CIN) or squamous intraepithelial neoplasia (SIL).[2,7] Not all cases of pre-malignant disease progress to invasive cancer.[9] The classification of the pre-malignant disease is based on the degree of abnormality of the epithelium ranging from mild dysplasia (CIN I) to severe dysplasia (CIN III).[10] Both low grade and high-grade dysplasia regress spontaneously in HIV negative women because the immunocompetent cells in the transformation zone of their cervix are adequate and confer on them a strong immune status.[11]

Probability of infection with wider variety of HPV types is higher in HIV-positive women without cytological abnormalities than in HIV-negative women.[12] In Nigeria, a population – based study conducted by Gage and Ajenifuja et al[13] in a rural community revealed that out of 1,282 non-virgins over age 25 years that attended the screening visit for whom HPV DNA test results were available, 14.7% were infected with one or more carcinogenic HPV genotypes.

Cancer of the cervix and HIV infection are major public health problems worldwide, more in Sub-Saharan Africa and especially Nigeria.[2,4,10] In the global ranking for the total number of people living with HIV, Nigeria is third with 1.7 to 4.2million people infected with the virus.[2] The risk of mortality from cervical cancer is higher in women infected with HIV.[5] There is also increased risk of having persistent infection with high risk human papillomavirus genotypes, reactivation of latent infection and development of progressive precancerous lesions in them because of impaired immunity.[7,12,13] In 2011, the Pink Ribbon Red Ribbon initiative which was a joint public–private international initiative was started with the aim of reducing cervical cancer in HIV-positive women.[10]

Cervical cancer is an AID-defining cancer according to United State Centers for Disease Control and Prevention classification and it runs a more rapid course in HIV positive women.[2,10] A large percentage of dysplastic lesions rapidly progress to more advanced disease in them. In HIV infection, the immune system is deregulated leading to a fall in the level of CD4 count, reversal of CD4:CD8 ratio and reduced Langerhan's cell counts.[13,14] Although the relationship between antiretroviral therapy (ART), HR-HPV and CIN seems to be complex, ART can restore the immune competence of the cervical mucosa by reducing the HIV plasma viral load (PVL) and therefore reduce the incidence of precursor lesions.[15]

Globally, the pattern of the cervical cytology among the HIV positive women is being studied and compared with their HIV negative counterpart especially in this era of HAART which may aid early identification and referral for proper treatment and follow up. In Nigeria where there is a large HIV epidemic and the impact of HIV on cancer on Nigerians especially people living with HIV/AIDS, there are not many studies done on this subject matter especially in the North Central region of the country. Because of few available data, there are impediments in the ability to formulate sound policy for clinical and public health intervention. Our centre provides HIV prevention and treatment services but do not have data on the prevalence of abnormal Papanicolaou (Pap) smear among the HIV women receiving care neither do we have a cancer register, hence the reason for this study. This study is therefore aimed at determining the pattern of cervical intraepithelial lesion amongst HIV positive women on HAART compared to their HIV negative counterparts at the Asokoro District Hospital, Abuja, Nigeria.

MATERIALS AND METHODS

The Study Location and duration: The study was conducted at the Asokoro district hospital between 1st of March 2020 to 31st of October 2020. The hospital is owned by the Federal Capital Territory, and it is in

the Asokoro district of the Abuja Municipal Area council. It is easily accessible from all parts of the territory and patients patronize the hospital services from all over the Federal Capital Territory. The average annual delivery rate of the hospital is over 2000 with a total bed capacity of 120. The hospital offers obstetric and gynaecological services to patients from all over the Federal Capital Territory and receives referrals from neighboring states including Nasarawa, Niger, Benue, Kaduna and Kogi states. Routine cervical cytology in the center is by Pap smear.

Study Design:

This was a prospective comparative cross-sectional study of Pap smear findings among HIV positive women on HAART and HIV negative women who assessed healthcare in the HIV, Gynaecological and the Family Planning clinics at Asokoro District Hospital, Abuja.

Study Population:

All the HIV positive clients were recruited from the HIV clinic while the HIV negative cohorts were recruited from the Family Planning and gynaecological clinics after proper counselling. All the subjects had documented evidence of their HIV status and written informed consent was obtained from them. Institutional ethical clearance was obtained, and confidentiality of clients was maintained.

Inclusion and Exclusion Criteria:

Women with positive HIV status on HAART for at least 6 months were recruited into the study. The exclusion criteria include HIV-positive and HIV-negative women who did not consent, had previous treatment for pre-invasive cervical lesion or cervical cancer, had a hysterectomy, have other coexistent immunosuppressive conditions (such as non-Hodgkin's lymphoma, Kaposi's sarcoma) or had immunosuppressive therapy and are pregnant or less than 6 weeks postpartum were excluded from the study.

Sample Size Calculation

The sample size was calculated using the formula for the calculation of sample size for two (2) independent proportions as follows:

$$N = \frac{2 (Z_{\alpha} + Z_{1-\beta})^2 x\ p(1-p)}{d^2}$$

Where,

N= Minimum sample size for each group
$Z\alpha$ = Percentage point of standard normal deviate (2 sided) set at 95% confidence level =1.96
$Z_{1-\beta}$ = Power of the test set at 80% (20% B error) = 0.84
P= Prevalence of abnormal cervical smears among HIV positive women from past study (34.4%) = 0.344[16]
d^2= Expected difference between the two groups = 0.15

$$N = \frac{2 (1.96+ 0.84)^2\ x\ 0.344(1-0.344)}{0.15^2}$$

$$= \frac{2 (7.84)\ x\ 0.225664}{0.0225}$$

= 157

A sample size of 157 was calculated for each arm of the study.

Sampling Technique

A systematic sampling method was used. The first participant from the clinic was randomly selected from the first K units in the population and every K^{th} element thereafter. K (the sampling interval) was determined by the formula

$$K = n/N$$

Where,

n = Total number of patients attending the clinic per month
N = Minimum sample size for each group
For gynaecology clinic, K = 720/157 = 4.58 (approximately 5)

where n = 120 (average number of patients attending the clinic per month) x 6 (period of sample collection) =720 and N is the minimal sample size = 157
For Family planning clinic, K = 1500/157 = 9.55 (approximately 10)
where n = 250 (average number of patients attending the clinic per month) x 6 (period of sample

collection) = 1500 and N is the minimal sample size = 157
For HIV clinic, K = 1680/157 = 10.7 (approximately (11), where n = 280 (average number of patients attending the clinic per month) x 6 (period of sample collection) = 1680 and N is the minimal sample size = 157

Data Collection Instrument

Women who met the inclusion criteria were counseled. The study topic and importance were explained to them in details, the benefits of the study were highlighted and stressed in a language that the women understood well. The copy of the consent form was given to the women who wish to participate in the study for their signature or thumb printing. A questionnaire was administered to the women by the investigator. The clinical and socio-demographic data were gotten from the structured questionnaires administered and participant's case note. Blood samples and cervical smear were collected from each of the study groups between 1st of March 2020 to 31st of October 2020. Confidentiality of cases was maintained by generating a serial enrolment number for each participant.

Data Collection Procedure

Blood samples were collected from each participant in the HIV positive group into the EDTA bottles and sent to the laboratory within 6 hours of collection for viral load analysis using polymerase chain reaction (PCR) and CD4 count analysis was done by flow cytometry using Cyflow. Counter II. The HIV testing for the HIV negative group was done using Determine HIV-1/2.

Each participant in both group was placed in dorsal position. A bivalve speculum was passed to expose the cervix. Gross inspection of the cervix was done, and findings were documented. Samples of cervical smear were collected by placing the Ayre's spatula firmly on the cervix, with the projection of the spatula within the cervical canal and rotated 360 degrees. Smears were placed on pre-labeled frosted slides, 2 slides per participant, based on the serial enrolment number for the particular participant. The slide was fixed immediately in 95% alcohol and transported to the laboratory in a coupling jar. The slides were stained using Papanicolaou staining technique. They were then stained with haematoxylin and decolorized with 95% alcohol solution, rinsed with water and stained with Orange G stain. They were then decolorized with 95% alcohol, stained with eosin azure 50. The histopathologist who read the slides was blinded to the HIV status of the participants.

Sample Handling

The values obtained were charted immediately against each participant's serial number. All data collected from the study were recorded appropriately and thereafter keyed into the Statistical Package for Social Sciences (SPSS) computer software version 21.

Data Analysis

Data was analyzed using the Statistical Package for Social Sciences software (SPSS) version 21. Descriptive statistics were used where appropriate. Chi Square test and Independent t-test were used to compare the results of cervical cytology between HIV seropositive and seronegative women. Level of significance was set at $P<0.05$ for analysis. Cytology results were categorized as negative, inflammatory, AGUS, ASCUS, LSIL and HSIL according to Bethesda classification of 1991.[17]

Ethical Consideration

Ethical clearance for this study was obtained from the Health Research Ethics Committee of the Health and Human Services Secretariat of the Federal Capital Territory, Abuja before the commencement of the study. Informed written consent was obtained from each participant after adequate counseling and the data obtained from the study was treated with confidentiality and used solely for the purpose of the study and any publications arising from the study. The women were offered the option to opt out of the study, bearing in mind such action would not in any way compromise the quality of care they would receive at any service point within the Asokoro district hospital.

RESULTS

A total of 314 women participated in the study, made up of 157 HIV positive and 157 HIV negative women (control). The demographic characteristics are shown in **Table 1**. The mean age of all the participants was 39.46±9.33 years. There was no significant difference between the mean ages of the two groups [39.82

(8.38) and 39.11(0.20) years, p=0.50]. Majority of the participants among the HIV positive group were traders, 79 (50.3%) while they are mainly civil servants in the HIV negative group. Largest proportion of the participants among the HIV positive group had secondary level of education, 65 (41.4%) while majority of them in the HIV negative group had tertiary level of education, 117 (74.5%). It can also be seen from the table that 90(57.3%) of the HIV positive and 117(74.5%) of the HIV negative group respectively were married.

Table 1: Socio-demographic characteristics of respondents

Variables	HIV positive n=157	Percentage (%)	HIV negative n=157	Percentage (%)	P value
Age					
20-30	23	14.7	38	24.2	
31-40	69	43.9	58	36.9	
41-50	53	33.8	38	24.2	0.60
51-60	11	7.0	22	14.0	
61-70	1	0.6	1	0.6	
Religion					
Islam	17	10.8	26	16.6	
Christianity	140	89.2	131	83.4	0.14
Educational level					
None	10	6.4	5	3.2	
Primary	32	20.4	10	6.4	
Secondary	65	41.4	25	15.9	0.00*
Tertiary	50	31.8	117	74.5	
Occupation					
Civil servant	32	20.4	89	56.7	
Farmer	14	8.9	3	1.9	
Trader	79	50.3	35	22.3	
Unemployed	27	17.2	24	15.3	0.00*
Student	5	3.2	6	3.8	
Marital status	90	57.3	117	74.5	
Married	31	19.7	38	24.2	
Single	10	6.4	1	0.6	0.00*
Divorced	26	16.6	1	0.6	
Widow					

*significant.

Table 2 shows the Pap smear results among HIV positive women. Those who had negative result were 139 (88.53%) while 15 (9.55%) had inflammatory pap smear result, 1 (0.64%) had LSIL, 1 (0.64%) had ASCUS and 1 (0.64%) also had AGUS in this group. None had HSIL.

Table 2: Pap Smear Results among HIV Positive women n=157

Variables (Pap smear result)	HIV positive n	Percentage (%)
Negative	139	88.53
Inflammatory	15	9.55
LSIL	1	0.64
HSIL	-	-
AGUS	1	0.64
ASCUS	1	0.64

Table 3 shows the Pap smear results among HIV negative women. Those who had negative result were 141 (89.8%) while 14 (8.92%) had inflammatory pap smear result. Only one respondent each had LSIL and ASCUS. **Table 4** shows the comparison of the Pap smear results of both groups. The proportion of normal findings

Table 3: Pap Smear Results among HIV Negative women n=157

Variables (Pap smear result)	HIV negative n	Percentage (%)
Negative	141	89.8
Inflammatory	14	8.92
LSIL	1	0.64
HSIL	-	-
AGUS	-	-
ASCUS	1	0.64

Table 4: Comparison of Pap Smear Results among HIV Positive and HIV Negative Women

Variables (Pap smear result)	HIV positive n=157	(%)	HIV Negative n=157	(%)	P value
Negative	139	88.53	141	89.8	
Inflammatory	15	9.55	14	8.92	
LSIL	1	0.64	1	0.64	
HSIL	-	-	-	-	0.88
AGUS	1	0.64	-	-	
ASCUS	1	0.64	1	0.64	

Table 5: Pap Smear Results and CD4 Count among HIV Positive Women n=157

Pap smear result	CD4 <200cells/ml	(%)	CD4 >200 cells/ml	(%)
Negative	1	100	138	88.46
Inflammatory	-	-	15	9.62
LSIL	-	-	1	0.64
HSIL	-	-	-	-
AGUS	-	-	1	0.64
ASCUS	-	-	1	0.64

Table 6: Pap Smear Results and Viral Load among HIV Positive Women n=157

Pap smear result	Viral load ≤20(%)	Viral load >20(%)
Negative	115(87.1)	23(92.0)
Inflammatory	15(11.4)	1(4.0)
LSIL	1(0.8)	-
HSIL	-	-
AGUS	1(0.8)	-
ASCUS	-	1(4.0)

(139; 88.53%) among HIV positive women on HAART approached that in the HIV negative women (141; 89.8%). Abnormal Pap smear results (n=5; 1.6%) was higher in HIV positive group (n=3; 1.9%) compared to the HIV negative group (n=2; 1.3%) but the difference in all the categories of cervical cytology abnormalities between the HIV positive group and the HIV negative group was not statistically significant (p= 0.88). None in both groups had HSIL.

Table 5 shows the Pap smear results with the CD4 counts amongst the HIV positive participants. It was found that 156 (99.4%) participants had CD4 counts greater than 200cells/ml while 1 (0.6%) participant had CD4 count <200cells/ml. The participants that had LSIL, ASCUS and AGUS were found to be among the women considered immune-sufficient (CD4 cell count >200 cells/ml).

Table 6 shows the Pap smear results with the viral load amongst the HIV positive participants. It was found that 25 (15.9%) participants had viral load greater than 20copies/mm^3 while 132 (84.1%) participants had viral load ≤20copies/mm^3. The participant with LSIL and that with AGUS have viral load of ≤20copies/mm^3 while the participant with ASCUS had a viral load >20copies/mm^3. None had HSIL.

DISCUSSION

The clinical course of HPV and SIL in HIV-related immunosuppression has been demonstrated to be changed by increase in the likelihood of viral persistence and lesion progression, respectively.[18-20] From this study, the proportion of normal findings (139; 88.53%) among HIV positive women on HAART approached that in the HIV negative women (141; 89.8%). This study has not shown significant difference in the prevalence of cervical cytology abnormalities between HIV positive group (n=3; 1.9%) and HIV negative group (n=2; 1.3%) at P value of 0.88 or P>0.05 which is similar to the findings from some studies but in contrast to some others that found higher prevalence among the HIV positive group like 26.6% reported by Lawal et al in Abuja, 34.4% reported by Bassey et al in Port Harcourt, 10.9% reported by Anorlu et al in Lagos, 31.3% reported by Chama et al in Maiduguri and 68.0% reported by Agaba et al in Jos, Nigeria, respectively.[4,10,21-23] Akarolo et al in their study on cancer burden among HIV-positive persons in Nigeria carried out a linkage study among a cohort of 17,826 persons living with HIV registered at health facilities where the Institute of Human Virology Nigeria (IHVN) provides HIV prevention and treatment services between 2005 and 2012 concluded that the risk of cervical cancer was not significantly increased in HIV infected women than in the general population.[24] This is similar to the result of this study carried out in our facility in which the HIV positive patients attending the clinic receive free and regular treatment services with good medication compliance.

Keller et al in their prospective study also found similar risk of cervical premalignant lesion and cancer in HIV- infected women and HIV-uninfected women and therefore concluded that HIV- infected women undergoing long- term clinical follow-up who are cytologically normal and oncogenic HPV–negative have a risk of cervical premalignant lesions similar to that in HIV- uninfected women.[18]

The CD4 count among the HIV-positive women that participated in the study is quite high ranging between 227 and 1866. The participants that had LSIL, ASCUS and AGUS among the 157 HIV-positive women in this study were among those with higher CD4 count which agrees with the findings of Coelho et al. who evaluated 115 HIV-positive women and discovered that those with LSIL and HSIL are found in those with higher CD4 counts (201–499) although not statistically significant. Zimmermman et al also reported no difference in CD4+ T-lymphocyte count in the presence or absence of LSIL or HSIL. Enebe et al [25] conducted a study in Enugu, Nigeria aimed to determine the association between low cellular immunity of HIV positive women, the prevalence and the grade of cervical squamous intraepithelial lesions concluded that the prevalence of cervical intraepithelial lesions among HIV positive women on HAART in Enugu, Nigeria is low and has no significant relationship with their CD4 cell count. In other words, the presence of lesions was not associated with immunosuppression.[19] The time of initiation of HAART, the viral load, CD4 count and PAP smear findings at the point of initiation of

HAART including the duration of HAART is important in women living with HIV as some may have high viral load, low CD4 count and some might have acquired the HPV infection and the premalignant lesion before commencing HAART.[20,26,27] The PAP smear result of these HIV positive women at the initiation of HAART are not known. HAART helps in the clearance of HPV infection by improving the patient's immune status, decreases the incidence and prevalence of SIL and reduces the risk of progression to high grade lesion in those with low grade lesion.[26]

HIV patients are immunocompromised, the immune system is deregulated leading to a fall in the level of CD4 count, reversal of CD4:CD8 ratio and reduced Langerhans cell counts making them more susceptible to human papilloma virus infection especially the high risk group with persistent infection leading to dysplasia which, if untreated, can lead to cervical cancer [13,14] but antiretroviral therapy can restore the immune competence of the cervical mucosa by reducing the HIV plasma viral load (PVL) and therefore enhance the clearance of human papilloma virus infection and reduce the incidence of precursor lesions.[15] Majority of the HIV positive participants in this study have a very good viral suppression and high CD4 count with possibility of an increase in their immune competence which might have contributed to the probability of cytologic regression and reduction in the risk of premalignant lesion in them comparable to that seen in the HIV negative arm. Notwithstanding, HIV-infected women on HAART must still receive careful gynaecologic follow-up and close routine monitoring.[27]

The result of studies on the effect of ART on the prevalence of premalignant lesions in the people living with HIV is inconsistent .[18,19,20,27] While some demonstrated a positive impact, some did not. Some studies done in Sub-Sahara Africa show significant impact of HAART on the development and progression of cervical lesion in the HIV positive women including the systematic review by Menon et al[20] on associations between highly active antiretroviral therapy and the presence of HPV, premalignant and malignant cervical lesions in sub-Saharan Africa. They suggest a positive impact of HAART duration, in conjunction and interaction with CD4 count, on reducing the prevalence of HR-HPV with maximum effect seen in women starting at the lowest CD4 count, which may have a more instrumental role in cervical oncogenesis than either HAART use or the treatment duration on the prevalence of moderate and severe cervical dysplasia.[20]

It has been confirmed that the use of highly active antiretroviral therapy (HAART) in HIV-infected individuals has a great effect on immune status.[27] Ahdieh-Grant et al. in their study on highly active antiretroviral therapy and cervical squamous intraepithelial lesions in human immunodeficiency virus-positive women discovered that HAART use was associated with increased regression of SIL among HIV-infected women and among women who used HAART, increased CD4 T-cell counts were associated with a greater likelihood of regression.[27] Despite these findings, there is a need for a long term follow up for the HIV positive women on HAART. A prospective cohort study found that within 3 years 1 in 5 HIV positive women with no evidence of cervical disease developed biopsy-confirmed squamous intraepithelial lesions.[22]

In this period of molecular testing, inclusion of HPV testing would most likely improve cervical cancer screening in HIV- infected women. There is evidence to suggest that HIV-positive women without cytological abnormalities may be infected with a broader range of HPV types than HIV-negative women.[12] This could not be done in our facility due to unavailability of the service and the cost implication among others.

CONCLUSION

This study has not demonstrated higher cervical cytology abnormalities in HIV positive women on HAART compared to HIV negative women assessing healthcare services in our hospital. Therefore, screening women living with HIV on HAART with very good viral suppression, high CD4 count and normal PAP smear result at 3- year intervals similar to HIV negative women seems safe as against the traditionally recommended annual screening by some treatment guidelines. This in addition may assist in reducing the cost of care and possibly also reduce the stigma connected to annual screening.

Limitation

The limitation of this study is that it did not look at the HPV testing and genotyping in both group of subjects. Histology was also not done.

Recommendations

Though the result of this study did not show high cervical cytology abnormalities among HIV positive women compared to their HIV negative counterparts, yet it will still be recommended that routine Pap smear screening should be integrated to the treatment packages for the HIV positive patient. This is because women generally have somewhat poor attitude and adherence to Pap smear screening. Study participants should therefore be encouraged to enroll under National screening program. It will be important that every HIV positive woman should have Pap smear done and documented alongside their CD4 count and viral load at the point of commencement of HAART. A three yearly Pap smear screening similar to HIV negative women seems to be safe in low risk women living with HIV on HAART.

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