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RESEARCH ARTICLE

ASSOCIATION BETWEEN VIRAL LOAD AND ABNORMAL PAP SMEAR IN HIV POSITIVE PREGNANT WOMEN AT A TERTIARY HOSPITAL IN EBONYI STATE, NIGERIA

*Chinomnso Stella Marcel-Onwudiwe, Assumpta Nnenna Nweke, Chidi Ochu Uzoma Esike, Amuchechukwu Veronica Nwafor, Emmanuel Chijioke Uwakwe and Uzoma Ede Ogbonna-Onwukwe

Department of Obstetrics and Gynaecology, Alex Ekwueme Federal University Teaching Hospital, Abakaliki; Nigeria

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ABSTRACT

Background: Cervical cancer is the commonest genital tract cancer worldwide, and has a high burden of morbidity and mortality in developing countries of which Nigeria is part of. Human immune deficiency virus (HIV) increases the risk of dysplastic changes in the cervix leading to increasing premalignant and malignant lesions of the cervix. Pregnant HIV positive women may be at a higher risk of having pre-invasive cervical lesions. Pregnancy presents an opportunity to screen women of reproductive age for the premalignant lesions of the cervix using Pap smear. **Objectives:** The objective of this study is to study the association between the cytological patterns of Pap smear of pregnant women who are HIV positive and the viral load levels at the antenatal clinic of Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA). **Methodology:** This is a cross-sectional study that involved collecting Pap smear from consenting HIV positive pregnant women attending antenatal clinic of AE-FUTHA. One hundred pregnant HIV positive women who fulfilled the inclusion criteria and gave consent were recruited into the study by consecutive sampling method. A pretested questionnaire was administered to the women to get needed information for the study. Papanicolaou smear test was done on each of the participants. Blood samples were collected from the antecubital veins of the forearms of the HIV positive women and sent to the laboratory for estimation of viral load. The data were analysed using Epi info version 7.2.1. The continuous variables were presented as mean and standard deviation while categorical variables were presented as numbers and percentages. Categorical variables were analysed using Chi square (Fisher's exact test was also used), while means were compared using t-test. A P-value less than 0.05 was considered as statistically significant. **Result:** The prevalence of abnormal Pap smear among HIV positive pregnant women was 24%. There was no relationship between viral load and abnormal Pap smear for HIV positive women with viral load less than 1000 copies per ml. **Conclusion:** This study has demonstrated no association between viral load and abnormal smear in HIV positive pregnant women.

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INTRODUCTION

Cervical cancer is the fourth commonest cancer among women worldwide and especially common in low and middle income countries^{1,2}. It is the commonest genital tract cancer in women in developing countries²⁻⁴. It is associated with high morbidity and mortality⁴. Worldwide, CA cervix accounts for about 570,000 new diagnosis and 311,000 deaths yearly^{5,6}. Approximately, 85% of deaths from cervical cancer occur in the underdeveloped countries and death rate is 18 times higher in the low income and middle income countries than in the higher income countries⁷. In Nigeria, about 12,000 new case of cervical cancer are recorded annually with about 8000 deaths⁸. Most of these morbidity and mortality caused from cervical cancer in developing countries are due to lack of proper

cervical cancer screening programs for women. Awareness and uptake of cervical cancer screening among Nigerian women including health workers, is very low^{9,10}. Moreover, there are no established screening programs in our environment especially in rural areas due to poverty, lack of resources and marginalization of women^{10,11}. Cervical cancer is usually preceded by the curable premalignant lesions; a precancerous condition, in which abnormal cell growth occurs on the surface lining of the cervix or endocervical canal^{11,12}. These premalignant lesions are also called cervical intraepithelial neoplasia (CIN) and include CIN I which is mild dysplasia, CIN II which is moderate dysplasia and CIN III which is severe dysplasia¹³. Several studies done on CIN in pregnancy has shown that pregnancy has no effect on cervical neoplasia while others believe that it regresses postpartum¹³. The incidence of abnormal cervical cytologic findings during pregnancy is 0.72 to 1.67%¹⁴. In the United States, about 2-3million abnormal Pap smear is diagnosed each year and 5%

to 13% of these are detected in pregnancy¹³. A study in Northern Nigeria, showed the prevalence of cervical dysplasia in pregnancy of between 4.8- 6%¹³. In Abakaliki, the prevalence of pre-invasive cervical cancer among pregnant women was 6.3%³. The peak incidence of CIN 1 and 2 were found in women in their third decade which is the time of maximal child bearing capacity¹⁵. This Cervical dysplasia usually has no symptoms and is often discovered by routine Pap smear test. The prognosis is excellent for women with cervical dysplasia and received appropriate treatment and follow-up^{15,16}. Cervical dysplasia and cervical cancer are caused by human papilloma virus (HPV) infection. Risk factors associated with cervical cancer and cervical dysplasia include; early coitarche, multiple sexual partners, having partner with multiple sexual partners, cigarette smoking, HIV infection, oral contraceptive use amongst others^{16,19}. Abnormal Pap smear and invasive cervical pathology as shown by a lot of scholarly articles are higher among HIV positive women compared to their HIV negative counterparts^{18,20,21}. Most HIV positive women are on highly active anti-retroviral therapy (HAART) which will help to improve their life span long enough for the development of CIN which could progress to invasive cancer if left untreated²². Although literatures have documented an association of HIV infection with a greater likelihood of development of cervical dysplasia/invasive cancer, little is known about the association between viral load level with the development of abnormal smears. Pregnancy presents a unique opportunity to screen women of reproductive age for cervical cancer and premalignant lesions of the cervix which is relatively common in this age group²³. Pregnancy may present a window of opportunity for pregnant women who have not had cervical cancer screening in the past. Association between the viral load levels and the development of abnormal smear is being studied as this will strengthen all avenues geared towards reduction in the viral load levels.

MATERIALS AND METHODS

Study location and duration: The study was carried out at the department of Obstetrics and Gynaecology in conjunction with the histopathology and haematology departments of AEFUTHA, Ebonyi State, Nigeria on the effect of viral load on Pap smear among HIV positive pregnant women attending antenatal clinic. The state has several health institutions of different cadres but with mainly primary health care facilities in the rural areas without facilities for screening for cervical cancer. There is also poor road network in the rural areas which may become inaccessible during the rainy season, making accessibility to health care facilities in the urban area with diagnostic tools difficult. AEFUTHA is a health institution formerly called Federal Teaching Hospital Abakaliki (FETHA). It is located at the center of the capital territory and receives referrals from all parts of the state and neighbouring states of Abia, Benue, Cross River and Enugu. The hospital has heavy obstetrics patient load as it caters for the primary, secondary and tertiary health needs of the state and surrounding states due mainly to the collapse of these cadre of health facilities.

Study design: The study was a comparative cross-sectional study carried out at the department of Obstetrics and Gynaecology AEFUTHA in conjunction with the histopathology and haematology departments of AEFUTHA among HIV positive and HIV negative pregnant women attending antenatal clinic.

Study population: HIV positive pregnant women on HAART.

Inclusion criteria and Exclusion criteria: Consenting HIV positive pregnant women on HAART were recruited into the study. The exclusion criteria included; pregnant women with history of cervical cancer, those previously treated or being treated for premalignant lesion of the cervix, pregnant women who were screened for cervical cancer in less than one year or those who have received HPV vaccine, age less than 21 years, women with history of premature rupture of membrane, women with history of antepartum haemorrhage or threatened miscarriage, pregnant women with abnormal vaginal

discharge, pregnant women in their first trimester, women with visible lesions.

Sample size: The sample size was determined using the following formula²⁴

$$n = z^2 pq/e^2$$

Where n = minimum required sample size

z = standard variant (1.96),

P = prevalence of abnormal Pap smear among HIV positive pregnant women in Abakaliki³

= 6.3% = 0.063

q = (1-p), = 0.937

e² = Acceptable error at 0.05.

= 1.96²*0.063*0.937/0.05²

= 90.71

Ten percent attrition rate was added to the minimum sample size.

Sample size = 100 per arm

Blood test: Blood sample were collected from all participating clients that were HIV positive for measurement of viral load. Three milliliters of blood were collected from their veins, using plastic disposable syringe for viral load. Results of those whose viral load were less than 6 months were copied from their folder, while those whose viral load results were more than 6 months had theirs repeated. This was done on the same day of the collection of cervical smears. Each sample for a particular client was coded for easy identification. HIV-RNA viral load was determined by polymerase chain reaction (PCR) and results were expressed as the number of RNA copies/ml.

Papanicolaou smear: Women were asked to avoid coitus, douching or vaginal insertion that will interfere with the result, 48 hours prior to the day of the test during counseling. The patients were subjected to both speculum and pelvic examinations to detect any gross abnormal lesions that would exclude them from the study such as detecting any abnormal vaginal discharge. General and abdominal examinations were also performed. These were done during booking and regular antenatal visits. The participants were placed in the lithotomy position and a sterile disposable Cusco speculum was passed into the vagina to examine the cervix under good light source. Ayre's spatula was inserted into the cervix and rotated 360 degrees. All samples were placed on two pre-labeled slides and a smear was made and immediately fixed by dipping the slide inside 95% alcohol and sample was sent to histopathology laboratory for staining with Papanicolaou staining technique. The fixed slides were hydrated in 90% and 70% alcohol each for 1 minute respectively. They were rinsed in water two times, and then stained first in Harris alum haematoxylin for 5 minutes. They were rinsed in water, then decolorized with 1% acid alcohol for 10 seconds and Blue in Scott's tap water for 5 minutes. They were rinsed in 95% alcohol again and then stained in orange G stock solution for 4 minutes. Then rinsed in 95% alcohol and finally stained with Eosin Azure 50 for 4 minutes. The slides were further rinsed with 95% alcohol. Cleared in xylene two times, 3 minutes each and mounted in a neutral synthetic resin medium and cover slip. The cytopathologist prepared and examined the slides. The cytopathologist was blinded to the HIV status of the patients. The Pap smear was reported using Bethesda 2001 system terminology. Women with abnormal smear were referred to the antenatal clinic for further examination. Abnormal Pap smears are; ASCUS, LGSIL and HGSIL, while the normal Pap smear are the normal smear and the inflammatory cells.

RESULTS

The socio-demographic characteristics are shown in table 1. There was no statistical difference in all the sociodemographic variables between the two groups. Mean age of the HIV positive women is 29.7 ± 5.5 years. Mean age of HIV negative women is 30.6 ± 4.5 years. The difference in the age was statistically not significant, P-value= 0.752.

Table 1. Sociodemographic Characteristics

Parameters	HIV positive	%	HIV negative	%	p-value
Age in years					
21-30	52	52.0	49	49.0	0.752
31-40	44	44.0	51	51.0	
41-50	4	4.0	0	0.0	
Total	100	100.0	100	100.0	
Mean age	29.7±5.5		30.6±4.5		
Religion					
Christianity	100	100.0	100	100.0	>0.999
Islam	0	0.0	0	0.0	
Others	0	0.0	0	0.0	
Total	100	100.0	100	100.0	
Occupation					
Civil servant	37	37.0	42	42.0	0.972
Trading	22	22.0	26	26.0	
Farming	6	6.0	2	2.0	
Artisan	10	10.0	4	4.0	
Student	17	17.0	16	16.0	
Unemployed	8	8.0	10	10.0	
Total	100	100.0	100	100.0	
Educationalstatus					
None	9	9.0	2	2.0	0.08
Primary	9	9.0	7	7.0	
Secondary	27	27.0	31	31.0	
Tertiary	55	55.0	60	60.0	
Total 1	100	100.0	100	100.0	
Parity					
0-1	59	59.0	67	67.0	0.141
2-4	28	28.0	26	26.0	
≥5	13	13.0	7	7.0	
Total	100	100.0	100	100.0	
Marital status					
Married	100	100.0	100	100.0	<0.999
Single	0	0.0	0	0.0	
Divorce	0	0.0	0	0.0	
Widow	0	0.0	0	0.0	
Total.	100	100.0	100	100.0	
Social class					
1	0	0.0	0	0.0	0.399
2	76	76.0	83	83.0	
3	13	13.0	12	12.0	
4	6	6.0	3	3.0	
5	5	5.0	2	2.0	
Total	100	100.0	100	100.0	

Table 2. Relationship between viral load and abnormal Pap smear result in HIV positive pregnant women

Pap smear result	Viral load undetectable (%)	Viral load <10 ³ (%)	Viral load 10 ³ -<10 ⁴ (%)	Total (%)	X ²	P-value	Post Hoc test p-value
ASCUS	4 (21.1)	9 (47.4)	6 (31.6)	19	4.134*	0.454	0.534
LGIL	0 (0.0)	1 (25.0)	3 (75.0)	4			0.339
HGIL	0 (0.0)	1 (100.0)	0 (0.0)	1			>0.999

*Fisher's exact test was used

All the respondents weremarried and they were all Christians. Majority of the respondents belong to social class 2, 70% and 83% for HIV positive and HIV negative respectively. Greater population of the participants are literate with 82.0% and 81.0% having secondary education or higher in HIV positive and HIV negative women respectively. Table 3 showed the relationship of the viral load with the Pap smear. Of 19 women that had ASCUS, 4 (21.1%) had undetectable viral load, 9 (47.4%) had viral load between 1 and 1000. Six (31.6%) had viral load greater than 1000. Of 4 women that had LGSIL, 1 (25.0%) had viral load between 1 and 1000, 3 (75.0%) had viral load greater than 1000. One participant who had HGSIL, had viral load between 1 and 1000. The relation between viral load and abnormal Pap smear is statistically not significant with P-value of 0.454

DISCUSSION

The importance of Pap smear in prevention of cervical cancer cannot be over emphasized. This study compared the association of viral load

and abnormal Pap smear result in HIV positive pregnant women on HAART. The prevalence of abnormal Pap smear of 24% among HIV positive pregnant women found in this study is similar to 25.2% found in Abakaliki by Agboeze et al.²⁵ This may be due to the fact that both studies were done in the same environment. It is higher than 12.6% found in Kolhapur, India by Madan et al²¹. The participants in Kalhapor were not pregnant women. This difference may be due to the fact that abnormal cervical cytology is higher in pregnant women than in non-pregnant women. However, the prevalence is less than 32.7% found in Kano by Muhammed et al²⁰. This difference may be due to the fact that most of the participants in Kano (60.0%) were either single, divorced or widow. These groups of women are more likely to have multiple sexual partner which is one of the risk factors of CIN while our respondents were all married. It is also less than 28.2% found in Nnewi by Ugboaja et al⁴. The difference might be due to difference in the age of the participants. The mean age of our participants was 29.7 while the mean age of participants in Nnewi was 37.7. Also some of the participants at Nnewi were not on highly active anti-retroviral therapy (HAART). This study showed no association between viral load with abnormal Pap smear. This

contrast general conception that abnormal Pap smear may increase with high viral load. However, all our respondents were on HAART and their viral load had reduced, some of them also had undetectable viral load and high CD4 count. Our findings between the viral load and abnormal smear was not statistically significant. This is at variance with what was found at Kano²⁰ which showed relationship between viral load and Pap smear. This difference may be due to the fact that our participants were pregnant women while participants at Kano were none pregnant women. In addition, most participants that had abnormal Pap smear in Kano, had viral load >10000copies/ml unlike our participants who were all on HAART and most of them had low viral load. The highest viral load among our participants that had abnormal Pap smear was <2000copies/ml, probably they had these lesion before they were commenced on HAART. Study done at Helsinki by Aho et al showed, decreased prevalence of CIN with controlled HIV infection and that Pap smear related risk factor is viral load >1000copies²⁶. Most of our respondents, had viral load <1000copies/ml. Our respondents' HIV infections were well controlled and may be the reason for ASCUS being the most prevalent, probably because the lesions were regressing.

CONCLUSION

This study has demonstrated the presence of premalignant cervical lesions in pregnant women with a significantly higher prevalence among HIV positive pregnant women. Therefore, there may be need to incorporate Pap smear test among routine antenatal investigations especially for HIV positive pregnant women.

RECOMMENDATION

Consequent upon the findings of this study, it will be beneficial to use pregnancy as a window of opportunity to screen women for premalignant lesion of the cervix with emphasis on HIV positive women. Also further studies on this area with multi-centre study may be needed.

Strength: This study was a comparative cross-sectional study which is stronger than cross-sectional study without comparison which was common among previous similar works among pregnant women. It utilized an important opportunity in reproductive age women to do the screening which many of them may not have done if they were not pregnant, as they may never present until the full blown disease manifests.

Limitation: The limitation of this study is that, being a hospital study, it may not be a true representation of what is obtainable in the general population. Also the physiological changes in the cervix during pregnancy may mask premalignant changes and some women with premalignant changes may have been missed. In addition, follow-up with biopsy and treatment is a challenge for screening positive women due to increased risk of haemorrhage.

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