

Original Research Article

High risk human papilloma virus **genotyping** and cervical cytology as screening tests for cervical cancer in human immunodeficiency virus positive women in Nigeria.

Abstract

Introduction

The integration of high risk oncogenic strains of human papillomavirus into the genome of cells in the cervix results in specific cytopathic changes. The propensity for dysplastic changes is worst in the presence of depressed immunity.

Aim: To compare screening methods by the identification of oncogenic strains of human papillomavirus (hrHPV) in cervical smears and the cytopathic changes among human immunodeficiency virus (HIV) positive patients.

Study design: Prospective cross-sectional study

Place and duration: Antiretroviral clinic and Cancer screening centre of the University of Port Harcourt Teaching Hospital, between 24th October- 30th of December, 2014.

Methodology. Eighty -two HIV positive **women** from the antiretroviral clinic with an equal number of HIV **negative women** were recruited as control. The presence of hrHPV **genotypes** were identified using real time polymerase chain reaction technique.

Cytological examination of cervical smears was done. The information obtained was processed using the SPSS version 20 software. Results are presented in tables, test validity computed, test of significance and association done using student's t-test and Odds ratio with P value < 0.05.

Results. HrHPV strains were more common among HIV infected **women** (P=0.03 OR 3.34 CI 1.03-10.84). High grade intraepithelial neoplasia was significantly associated with hrHPV (P=0.006,OR 14.75 CI 2.32-93.91). HrHPV **genotyping** as a screening test

26 was 88% specific with negative predictive value of 96.7% (CI 0.89-1.0) for determining
27 high grade intraepithelial lesions

28 Conclusion: Human immunodeficiency virus infected women are associated with higher
29 risk of acquiring highly oncogenic strains of HPV. High risk HPV genotyping with high
30 specificity and negative predictive values in identification of patients with dysplastic
31 changes should be used as adjunct to cytological screening in HIV positive patients.

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33 Keywords: high risk HPV, genotyping, cervical smears, Nigeria.

34 INTRODUCTION

35 A large proportion of gynaecological cancer related mortalities have been linked to
36 cervical cancer in Nigeria and other Sub-Saharan African countries [1,2]. In Asia, similar
37 epidemiology have been identified [3,4] while converse is the case in Nordic countries
38 where such mortalities have been reduced significantly by the adoption of organized
39 screening modalities [5,6]. This unfortunate occurrence in the developing countries had
40 been linked to the absence and lack of utilization of cervical cancer screening strategies
41 among the at risk population [7].

42 The HIV pandemics largely affect same population of persons within the geographical
43 entities associated with increased cervical cancer mortality [8,9]. The Nigerian statistics
44 indicates that about 3.5 million women are living with the human immunodeficiency
45 virus in Nigeria [10]. The presence of cervical cancer in patients with HIV had been
46 categorized as an AIDs defining disease by the Centre for Disease Control [11]. This
47 poses, a public health challenge because with the regular use of antiretroviral therapy,
48 the life expectancy of patients are prolonged bringing into view the effect of persistent
49 oncogenic strains of HPV and dysplastic changes culminating in increased incidence of
50 cervical cancer in this subset[12].

There are several **genotypes** of human papilloma virus with each exhibiting different oncogenic properties [13,14]. High risk strains of human papillomavirus (16,18,33,35,39,45,51,56,58,59,68) have been linked with a high propensity for cytological changes associated with moderate to severe dysplasia in the epithelial tissues, which are precursors to cervical cancer [15]. Spontaneous regression of the HPV infection is the norm in most patients [16], however persistence of these oncogenic strains in the cellular matrix results in integration of the HPV genome resulting in modulation of tumor suppressor proteins such as p53 and Rb culminating in cytopathic changes[17]. The persistence rate of high risk oncogenic strain in the cellular matrix have been shown to be **higher** in patients with HIV infection [18].

The practice in Nigeria and most developing countries for screening among these highly susceptible cohort for premalignant transformation is the use of the traditional papanicolaou Smear [19,20]. Since, late presentation and lack of utilization of screening modalities had been noted in majority of the population at risk,[8] it is imperative to look inward for methods that will narrow the population at risk and ensure early diagnosis; bearing in mind that 99% of patients with cervical cancer are associated with high risk human papilloma virus infection[13-15].

It is based on the above, that this study seeks to look at high risk human papillomavirus **genotyping** and the traditional PAP smear as screening methods for cervical cancer in women attending the antiretroviral clinic in Port Harcourt, South-South, Nigeria.

Methods/materials

Ethical clearance: Approval for the study was obtained from the Internal Review Board (IRB) of the University of Port Harcourt Teaching hospital before the commencement of the study with IRB number ADM/90/S11/VOL X 357.

76 A cross sectional prospective study on 82 human immunodeficiency virus positive
77 women from antiretroviral clinic of the University of Port Harcourt Teaching Hospital,
78 Port Harcourt between 21st October and December 2014 was conducted. All women
79 between 15 and 70 years were included in the study while women who had total
80 abdominal hysterectomy, failed to give consent for the study, treated for premalignant
81 lesion of the cervix, who were adequately immunized against the HPV, women receiving
82 antineoplastic therapy and those who are not eligible for papanicolaou smear such as
83 women who were menstruating, had intercourse in the last 48 hours, etc were excluded
84 from the study. An equal number of human immunodeficiency virus negative women
85 were recruited from the gynaecological/family planning clinics of the hospital as
86 control, applying same exclusion and inclusion criteria as the principal. The HIV status
87 of all patients from the gynaecological and family planning clinics were determined
88 using the “Determine” rapid diagnostic test kit (Alere medical co Ltd Japan), before
89 commencement of the research and only those identified as negative were recruited
90 into the study as control.

91 The sample size was determined using the Leshe–kish formula for single proportion
92 [21] with prevalence of human papilloma virus in women in Ibadan, Nigeria used as
93 reference prevalence[22]. Allowing a precision of 100%, the minimum study population
94 estimated was 73. A total of 82 persons each were recruited for the study.

95
96 After due counseling on the research, informed consent was obtained and patients were
97 recruited into the study using simple random sampling technique as they presented
98 until the required sample size was achieved.

99 The cervical smears were collected with the cytobrush for Papanicolaou smear and HPV
100 **genotyping**. In collecting the sample, the women were placed in lithotomy position, the

cervix was exposed using the bivalve disposable plastic speculum and then inspected. The cytobrush was introduced into the transformation zone scrapped in a clockwise manner to 360°. The specimen for the HPV genotyping was collected in the same manner using the cytobrush. After collecting the samples, the cytobrush was spun and the condensate was smeared on an already labeled frosted end glass slide. The smeared slides were stained using the papanicolaou staining method and stained slides were reported in the Anatomical Pathology Department of UPTH. The cytobrush for HPV serotyping was inserted into an already numbered specimen bottle, which contained a physiological saline solution. This was then cut short which allowed the specimen bottle to be covered. All the collected samples were packaged in an ice pack container and couriered to the Safety Molecular Pathology laboratory at Enugu, which was used for HPV DNA testing.

The samples were tested using a multiplex PCR method for the detection of 13 different high risk HPV genotypes namely: 16,18,31,33,35,39,45,51,52,56,58,59 and 61 using the High pure PCR preparation kit (Roche Diagnostics, United Kingdom)

In determining the HPV genotypes, a PCR mixture was prepared in a tube. The mixture contained the DNA sample (extracted from the cytobrush scrappings), primers Gp 168, my313 and OLis35 (Eurofin, Germany) and reaction buffer from magnesium chloride. Other contents of the mixture included deoxynucleosides triphosphates (dNTPs) and Taq DNA polymerase.

DNA-extraction: This was done using the DNA extraction buffer. The cytobrush was washed in 1.0ml of normal saline, vortex vigorously and centrifuged at 13000rpm for 15 minutes. The supernatant from the tube was removed and discarded. Thereafter, 1.0ml of normal saline was added and the pellet suspended with the vortex. This was centrifuged at 13000rpm for 5 minutes. The supernatant was again removed and

discarded. Then 50ul of the DNA extraction buffer was added and the tube and vortex closed for 10 seconds. This was spun down briefly in a table centrifuge. The tube was incubated for 10 minutes at 100 degrees centigrade and centrifuged at 13000rpm for 5 minutes. The supernatant, which contained the extracted DNA, was used for PCR template.

The aliquot mixture was placed into well plates and the plates covered with a microseal film. The plate was placed in a real-time PCR machine that ran a programme that has been set up earlier. The programme involved denaturation, annealing and extension/elongation steps. The initial denaturation was done by heating the mixture to a temperature of 95 degrees centigrade for 10 minutes. Further heating of the mixture for about 30 seconds at the same temperature carried out further denaturation. The annealing step was carried out by cooling the mixture to a temperature between 58 degrees centigrade for 45 seconds. The extension step was achieved by heating the PCR mixture to a temperature of 72 degrees centigrade.

The above steps were repeated 40 times. This resulted to exponential growth of the target DNA. DNA amplification was done based on the cleavage points on the E1, E6-E7 and L1 regions of the HPV genome. A billion copies of the DNA of the target fragment were created over 4 hours. The mixture was heated again to 92 degrees centigrade and cooled again to 55 to 72 degrees centigrade for 5 minutes in the final extension step. This step ensured that the remaining single strands are fully extended. The exponential/amplification to 40 times resulted in 68 billion copies. A hold of the mixture at 4 degrees centigrade for indefinite time limited the polymerase activity and made the sample ready for analysis of the HPV DNA genotypes. The PCR was carried out in a thermal cycler that heated and cooled the reaction tube, which achieved the required temperature at each step of the reaction.

Information obtained at the end of this study was processed using a computer Software Statistical Package for Social Science version 20.0 (SPSS Inc; Chicago USA). Frequency tables were generated and the results tested for significance using student t-test and chi-square. The validity of the screening tests was assessed by determination of the sensitivity, specificity and negative predictive values. The risk of acquiring high risk HPV was estimated with odds ratio. Statistical test of association was carried out at the level of significance set at P value <0.05 at 95% confidence interval.

Results

The prevalence of high risk human papillomavirus **genotypes** in HIV positive patients was 14.6% as against the control of 4.8%. (P=0.03, OR3.34 CI 1.03-10.84).

Among the HIV positive, **hr**HPV positive patients, 50%(6) had **genotypes** 18, 25%(3) **genotype** 16 while 16.7(2) and 8.3(1) had **genotypes** 33 and 35 respectively. Among the control 50% had **genotypes** 16 and 35 respectively.

Table 2 showed the correlation of hrHPV to cervical intraepithelial lesions. Among the hrHPV patients 66.7% (8) had normal cytology while 33.3% (4) had high grade squamous intraepithelial lesions while among the control 72%(59) had normal cytology 2.4% (2) each had LGSIL, HGSIL and **AGC** respectively while 6.2% had inflammatory changes on cytology.

Presence of high risk HPV was significantly associated with high grade squamous intraepithelial lesions (P=0.006,OR 14.75 CI 2.32-93.91)

The sensitivity of the HPV serotyping compared to cytology as a screening test for cervical cancer was 66.7% with a specificity of 88.1%. The positive predictive value was 33.3% (95% CI 0.09-0.65) with a negative predictive value of 96.7%(95% CI 0.89-1.0)

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Discussion

This study identified a three fold increase in the prevalence of high risk oncogenic strains of HPV among HIV infected women as against the control. This finding is in agreement with similar studies in Nigeria and other parts of the world which indicate higher risk [23-25]. Regional variations in the prevalence of HPV infections among HIV positive patient is influenced by socio-sexual risk factors and the place of antiretroviral therapy [12, 26]. The advent of antiretroviral therapy had prolonged the survival of HIV positive patients while making the occurrence of HPV related cervical cancer a public health challenge.

The oncogenic strains identified in the HIV positive group were HPV 18,16,33 and 35 in descending order of frequency as against that of the control, which identified genotypes of 16 and 35. The predominance of 18 and 16 tends to conform with the global

prevalence rates [27] as against findings by Nweke et al and sally et al in Nigeria [23, 28] who identified genotypes 31,35, 52 and 53 as predominant among HIV patients.

There is an up to 80% risk of acquisition of the HPV virus in any sexually active female during her reproductive lifespan [14] with most having spontaneous regression in the presence of adequate cellular immunity, however in HIV positive women local persistence and higher progression rates had been observed to be more common[29,30]. The impregnation of the HrHPV genome into the cervical epithelium facilitated by the higher persistence rate causes the cytopathic changes whose identification formed the basis for the traditional screening method developed by George Papanicolaou et al in the 1950[31].

This study showed that patients with hrHPV were observed to have a 15 fold increase in the risk of having high grade squamous intraepithelial neoplasia, compared to non HIV patients, which is a precursor with a greater risk of malignant transformation. This

transformation of the impregnated normal cellular matrix to high grade intraepithelial lesion takes an average of 10 -20years in non immune compromised patients as against that observed in HIV infected patients which takes a shorter duration.

The implications of the shorter duration of transformation and the obvious relations between hrHPV and cervical cancer makes it imperative for steps to be taken for early detection of at risk patients. In this study, it was observed that 66.7% of all oncogenic HPV positive patients had normal cytological features, which implies that cytologic screening alone may not be sufficient to pick patients who are at risk of developing cervical dysplasia/cancers. This observation was also reported by other investigators [32, 33]. The high negative predictive value and specificity of HPV genotyping in determining cytopathic changes noted in the study makes hrHPV genotyping an important tool in isolating those at less risk of the precursors of cervical cancer and thus allowing the lean resources of Government to be concentrated on those who are really at risk.

Thus any modality of screening that identifies the actual group of patients at risk should be encouraged at any opportunity, these women have contact with health care facilities creating an avenue for enforcement of the message of more vigilance for cervical cancer.

The lack of use of cervical cancer screening strategies by most Nigerians and the poor sensitivity of the traditional cervical cytological evaluation of patients brings to the front burner the need to adopt a screening method that encourages early surveillance especially in HIV infected patients which the HPV testing offers.

There is need to develop a national cervical cancer screening policy which should incorporate high risk hrHPV serotyping among high risk population instead of the commonly used traditional practice of PAP smear. The limitations of PAP smear poor sensitivity, need for a trained pathologist and inter-observer errors [34] makes it a

draw back as a sole screening modality among HIV positive patients as is currently practiced in Nigeria and most countries of the world.

This study is limited by the fact that the population studied was hospital based hence community based study is thus advised, which may be hindered by the cost of hrHPV genotyping in our environment.

In conclusion, the observations of Ronco et al [35] which identifies HPV screening ability to improve screening of cervical cancer compared to conventional Pap smear screening supports the observation of the authors for a possible change of screening modality among HIV positive patients. Adoption of this recommendation may reduce the economic burden on the health sector by narrowing down on the actual persons at risk and possibly prolonging the interval of PAP smear screening.

References

1. Anorlu RI, Obodo K, Makwe CC. Cancer mortalities among patients admitted to the gynaecological ward at Lagos University Teaching Hospital. *Int J Gynaecol Obstet.* 2010.110(3): 268-269.
2. Kamanger F, Dores GM, Anderson WF. Pattern of cervical cancer incidence mortality and prevalence across five different continents defining priorities in different geographical regions of the world. *J Clin Oncol.*2006. 24(14): 2137-50.
3. Sankaranarayanan R, Ramados K, Qiao Y. Managing the changing burden of cancer. *BMC Med.*2014.12.3: 1-17.
4. Aswathy S, Reshma J, Avani D. Epidemiology of cervical cancer with special focus on India. *Int J Women Health.*2015.7: 405-14.

- 250 5. Larra E, Dayn E, Hakama M. Trends in mortality from cervical cancer in the
251 Nordic countries: association with organized screening programmes.
252 Lancet.1987. 30.1(8544): 1247-9
- 253 6. Linday AT, Freddie B, Rebecca LS, Jacques F, Joanne L, Ahmedin J. Global cancer J
254 Clin 2015.65(2): 87-108.
- 255 7. Bassey G, Nyengidiki TK, Onwubuariri M. Profile and retrospective analysis of
256 the use of preventive strategies in patients with cervical cancer in South South
257 Nigeria. Nig Med J. 2015.56(2): 109-112
- 258 8. Chirenji ZM.HIV and cancer of the Cervix. Best Pract. Resp Clin Obstet Gynaecol.
259 2005.19(2): 269-76.
- 260 9. Holmes RS, Hawes SE, Toure P, Dem A.feng Q, Weiss NS, Kiviat NB.HIV infection
261 as a risk factor for cervical cancer and CIN IN Senegal. Cancer Epidemiol
262 Biomarkers Prev. 2009.18(9): 2442-6
- 263 10. Federal Ministry of Information and national orientation. The Obasanjo reforms:
264 HIV/AIDS response. Abuja 2007.
- 265 11. Centre for Disease control and prevention. Revised classification system for HIV
266 infection and expanded Case Surveillance definition for AIDS among Adolescence
267 and Adults.1993.MWWR. 14:1-19
- 268 12. Franceschi S, Jaffe H. Cervical cancer screening of women living with HIV
269 infection: a must in the era of antiretroviral therapy. Clin Infect Dis.200. 45 (4):
270 510-3.
- 271 13. Smith JS. HPV distribution in invasive cervical cancer and high grade cervical
272 lesions: A meta analysis update. Int J cancer.2007.121: 621-632
- 273 14. Baseman JG, Koutsky LA. The epidemiology of HPV infections. J clin Virol.
274 2005.32(1): S16-225.

- 275 15. Bosch FX, Lorinez A, Munoz N, Meijer CJ, Shan KV. The causal relationship
276 between HPV and cervical cancer. J Clin Pathol.2002.55(4):244-265
- 277 16. Moscicki AB, Shiboski S, Broering J. The natural history of HPV infection as
278 measured by repeated DNA testing in adolescent and young women. J
279 Paediatr.1998.132: 277-284.
- 280 17. Kwawukume EY, Srofenyoh EK. Premalignant lesions of the female genital tract.
281 In kwawukume, Emuvanyan (edn), Comprehensive Gynaecology in the Tropics.
282 1st edition. Accra. Graphic packaging ltd.2005: 396-411
- 283 18. Pooja P, Mala YM, Tripathic R, Das BC, Bhambrani S. Human papillomavirus
284 (HPV) infection and abnormal cytopathology among HIV positive women in
285 India. J AIDS HIV Res. 2014.6(1): 1-6
- 286 19. Oche MO, Kaoje AU, Gana G, Ango JT. Cancer of the cervix and cervical screening.
287 Current Knowledge, attitude and practices of female Health workers in Sokoto,
288 Nigeria. Int J Med Med Sci. 2013.5(4): 184-190
- 289 20. Wright TC Jr. Kuhn L. Alternatives to approaches to cervical cancer screening for
290 developing countries. Best Pract Clin Obstet Gynaecol.2012. 26(2): 197-208.
- 291 21. Jekel JF, Katz DL, Elmore JF. Sample size, randomization and probability theory.
292 Epidemiology, biostatistics and preventive medicine. 2nd edition Philadelphia.
293 WB Saunders 2001: 196-204.
- 294 22. Thomas JO, Herrero R, Omigbodun AA, Oje-Makinde K, Ajayi OI. Prevalence of
295 Papillomavirus infection in Ibadan, Nigeria. A population-based study. Br J
296 Cancer.2004. 90(3): 638-645

23. Nweke IG, Banjo AAF, Nwadike VU. Prevalence of Human Papilloma virus DNA in HIV Positive women in Lagos University Teaching Hospital (LUTH), lagos, Nigeria. *British Microbiology Research Journal*. 2013.3(3): 400-413
24. Nweke IG, Nwadike VU, Kalu IE, Ojide KC. Human Papilloma virus and cervical neoplasm in HIV positive women: A non systematic review. *Afr J Microbio Res*. 2014.8(22). 2193-2201
25. Anna-lise Williamson. The interaction between Human immunodeficiency virus and human papillomavirus in heterosexuals in Africa. *J Clin Med*.2015. 4(1): 579-592
26. Beatriz G, Valdilen GV, Jose EL, Luciane V, Paula ML, Ruth KF et al. Factors associated with increased prevalence of human papillomavirus infection in a cohort of HIV infected Brazilian women. *Int J infect Dis*. 2009.1(13):72-80
27. Sanjose S, Quint WGV, Alemany L, Geraets DTI, Klanstermeier JE, Lioveras B et al. Human Papillomavirus genotype contribution in invasive cervical: a retrospective cross sectional worldwide study. *Lancet Oncol*.2010.1.1048-56.
28. Sally NA, Maryam A, Ayotude OF, Eileen OD, Olayinka BO, Richard O, Costette MW, Clement AA. *BMC Infect Dis*.2013.13: 521-27.
29. Stratton P, Ciacco KH. Cervical neoplasm in the patients with HIV infection. *CurrOpinObstetGynaecol*.1994.6 (1): 86-91
30. Palefsky JM, Holly EA. Immunosuppression and co-infection with HIV. *J Natl Cancer Inst Monogr*.2003.31: 41-46
31. Siang YT, Yvonne T. George Papanicolaou (1883-1962): Discoverer of the Pap smear. *Singapore Med J*.2015.56 (10): 586-587.

32. Sawaya GF. Adding human papillomavirus testing to cytology for primary cervical cancer screening: shooting first before asking questions editorial. Ann Intern Med. 2008.148(7): 557-559
33. Deblinta DS, Koutsky LA, Rattele S, Unger ER, Shlay J, McClain T et al. Human papillomavirus infection and cervical cytology in women screened for cervical cancer in the United States of America 2003-2005. Ann Intern Med. 2008. 148(7): 493-500.
34. Nyengidiki TK. Screening for gynaecological cancers. In Darwish A (edn). Contemporary Gynaecological Practice. InTech Publishers, Croatia. 2015: 127-142.
35. Ronco G, Dillner J, Elfstrom MK, Tunesi S, Snijder PJT, Kitchener H et al. Efficiency of HPV- based screening for the prevention of invasive cervical cancer: follow up of four European randomized controlled Trial. 2014.383(9916):524-532.

TABLES

Table 1: Distribution of high risk HPV serotypes according to HIV status

HIV status	hrHPV status					Total hrHPV positive n(%)
	Negative n(%)	Positive n(%)				
		16	18	33	35	
Positive 82(100)	70(85.4)	3(3.7)	6(7.3)	2(2.4)	1(1.2)	12(14.6)
Negative 82(100)	78(92.7)	2(2.4)	-	-	2(2.4)	4(4.8)

344 Table 2: Correlation of hrHPV to cervical cytological lesions

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hrHPV status	Cytological changes n(%)						Total
	Normal	ASCUS	LGSIL	HGSIL	AGC	IC	
Positive	8(9.8)	-	-	4(4.8)	-	-	12(14.6)
Negative	59(72.0)		2(2.4)	2(2.4)	2(2.4)	5(6.2)	70(85.4)

346 NB: ASCUS-Atypical squamous cells of unspecified significance; LGSIL- low grade
 347 squamous intraepithelial lesion; HGSIL- High grade squamous intraepithelial neoplasia,
 348 AGC-Atypical glandular cells, IC -inflammatory cells