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Comparative Analysis of Cervical Human Papillomavirus DNA Testing and Cytological Findings among Women Presenting for "Pap" Smear in a Tertiary Health Centre in Northern Nigeria

M. M. Manga[1*], A. Fowotade[2], Y. M. Abdullahi[3], A. U. El-Nafaty[4], S. Adamu[5], A. D. Bojude[6], H. U. Pindiga[3], R. A. Bakare[2] and A. O. Osoba[2]

[1]Department of Medical Microbiology and Immunology, Federal Teaching Hospital Gombe, Gombe State, Nigeria.
[2]Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Oyo State, Nigeria.
[3]Department of Histopathology, Federal Teaching Hospital Gombe, Gombe State, Nigeria.
[4]Department of Obstetrics and Gynaecology, Federal Teaching Hospital Gombe, Gombe State, Nigeria.
[5]Department of Chemical Pathology, Federal Teaching Hospital Gombe, Gombe State, Nigeria.
[6]Department of Radiotherapy and Oncology, Federal Teaching Hospital Gombe, Gombe State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MMM, AF, YMA, AUEN and RAB conceived the study and drafted the manuscript. Authors MMM, AUEN and YMA were responsible for specimens and data acquisition. Authors MMM, AF and YMA were responsible for all laboratory analyses. Authors ADB and SA performed statistical analysis and data interpretation. Authors AOO, RAB and HUP participated in the design and coordination of the study. All authors contributed to data interpretation, read and approved the final manuscript.

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**Corresponding author: Email: drmmanga@gmail.com, mangamuhammad@yahoo.co.uk;*

ABSTRACT

Aim: This study was conducted to compare different cytological findings with cervical HPV infection among women presenting for cervical cancer screening in Gombe north-eastern Nigeria.
Study Design: It is a hospital based cross-sectional study.
Place and Duration of Study: Departments of Obstetrics/Gynaecology and Histopathology Federal Teaching Hospital Gombe (FTHG) Nigeria, between August 2013 and May 2014.
Methodology: Two hundred and nine (209) women were subjected to liquid-based cervical cytology and HPV DNA testing.
Results: Of the 209 participants, cytological findings were normal in 126 (61.6%) women while 80 (39.0%) had abnormal features. Three (1.4%) respondents had unsatisfactory smears. The observed abnormal cytological features include HPV changes 30 (14.4%), HPV changes with inflammation 2 (1.0%), inflammatory changes alone 36 (17.3%), Low Squamous Intraepithelial Lesion; LSIL 3 (1.4%), High Squamous Intraepithelial Lesion; HSIL 5 (2.4%) and malignant changes 3 (1.4%). Positive HPV DNA testing was detected among 100 (48.1%) of the participants. Almost half 60 (47.6%) of the women with normal cytology were positive for HPV. Among women with cytologically detected HPV changes, only 16 (50%) were also HPV DNA positive. The sensitivity and specificity of cervical cytology in detecting HPV infection was 16.2% and 85.0% respectively.
Conclusion: This study reports a very low sensitivity but relatively high specificity of cytology in detecting cervical HPV infection. It further justifies the need for introduction of HPV DNA testing to improve efficiency and maximise the sensitivity of cytology based cervical cancer screening for women above 30 years.

Keywords: Cervical cancer; cytology; human papillomavirus; DNA testing; Nigeria.

1. INTRODUCTION

Human Papillomaviruses (HPVs) have remained a serious global health problem due to their aetiologic association with anogenital/oral cancers and warts [1]. Persistent infection with HPV is the major risk factor for cervical cancer as more than 99% of all cases contain the high risk (hr) HPVs [2]. Of about 40 HPV types known to infect the uterine cervix, thirteen (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66) have been classified as group 1 carcinogens [3,4]. Beside being the commonest sexually transmitted infection (STI), infection of the genital tract with HPV is commoner among young sexually active women [5]. In some studies, up to 70% of college-aged women have been found to be HPV DNA positive. This probability increases with increasing number of lifetime sexual partners. Fortunately, most HPV infections in young women (below 30 years) are transient and it is only the small proportion of women who become persistently infected with hr-HPV that remain at risk for subsequent development of CIN or cervical cancer [6]. Therefore, an easy way to identify persistent HPV infection is to restrict screening (by HPV testing) to women aged 30 years or older [6]. Persistence has also been defined by positivity for the same HPV type following more than one DNA testing [7]. There is no recommended specific antiviral therapy to eradicate HPV infection. Treatment is directed mainly to the macroscopic or pathologic precancerous lesions caused by HPV.

Cervical cancer is the second most common cancer in women worldwide. It is the leading cause of cancer deaths in developing countries but gradually becoming a rare disease in many developed countries [8]. In Nigeria, cancer of the uterine cervix is the commonest genital tract malignancy in the northern region [9,10]. This has made studies on HPV in relation to cervical cancer screening even more important in the region.

Screening with cervical cytology at regular intervals has globally formed the basis for cervical cancer prevention [11]. The Papanicolaou (“Pap”) smear is a simple and well-accepted procedure for efficient detection of potentially premalignant HPV-associated cervical lesions through cytological examination of exfoliated cervical cells [12]. However, the need for repeated screening cycles makes cytology-based cervical cancer screening programmes expensive [4]. Moreover, this conventional screening model (Pap smear followed by colposcopically directed biopsy) is neither sufficiently developed nor sustainable in most

countries with limited resources [13]. The newer Liquid-based cytology (LBC) has replaced conventional Papanicolaou cytology as the platform for cervical cytology in a number of developed countries. Advantages of LBC include more rapid screening of slides, a substantial reduction in unsatisfactory cervical samples requiring repeat testing, and a cellular residue suitable for HPV testing [5]. However, even such improved cytology tests may miss 15% to 35% of CIN III or cancer in a routine screening setting [14]. Hence the importance of cervical HPV testing in cervical cancer screening.

In this study we compared cervical infection with oncogenic HPV types and the various cytological findings among women who presented for routine cervical cancer screening at the Federal Teaching Hospital Gombe (FTHG), North-eastern Nigeria.

2. MATERIALS AND METHODS

2.1 Study Design and Population

The study was an observational hospital based cross sectional study among 209 women who presented for cervical cancer screening (using LBC) at the FTHG between August 2013 and May 2014. Women who gave an informed consent for HPV DNA testing among those presenting for cervical cancer screening were counselled and recruited for the study. Ethical clearance was obtained from the research and ethics committee of FTHG.

2.2 Inclusion Criteria

All women who gave an informed consent for HPV DNA testing among those presenting for cervical cancer screening in FTHG.

2.3 Exclusion Criteria

Women who are menstruating or have vaginal bleeding at the time of specimen collection during the study period.

2.4 Specimen Collection, Preservation and Processing

Cervical cells were obtained using Rovers® Cervex-Brush® cell sampling device (Rovers Medical Devices B.V 5347 KV Oss, The Netherlands). The brush was subsequently transferred into the preservative fluid of a liquid-based cytology system; *Liqui-PREP* (LGM International, Inc, Melbourne, FL, USA).

DNA extraction for the PCR was done using proteinase K digestion, followed by phenol/ chloroform and ethanol precipitation method.

Polymerase Chain Reaction (PCR) for the detection of oncogenic HPV was done using the GP5+/GP6+ (GP5+ [5' TTTGTTACTGTGGTAGATACTAC-3'] and GP6+ [5'- GAAAAATAAACTGTAAATCATATTC-3']) consensus primers which amplifies a 150bp fragment of the L1 HPV genomic region [15]. AccuPower HotStart Premix (Bioneer Corporation, South Korea) was used for the PCR. Target amplicons were identified on a 2% agarose gel after electrophoresis.

Cervical cytological findings were reported by a cytopathologist using the 2001 Bethesda system independent of the HPV status of the participants.

Laboratory procedures were carried out at the departments of Histopathology FTHG and the DNA Labs, Kaduna, Nigeria.

2.5 Data Analysis

Statistical data analysis was done using the Statistical Package for the Social Sciences version 22 (SPSS Inc., Illinios, USA). Data entry in to the SPSS was done using numeric codes. Student's *t* test was used to derive mean, standard deviation and test of comparison for continuous variables. Categorical variables were summarized as proportions and further analyzed using Chi square to assess association between them. Sensitivity, specificity, Positive predictive value (PPV) and Negative predictive value (NPV) were calculated using the formulae; TP/TP+FN, TN/TN+FP, TP/TP+FP and TN/TN+FN respectively. Tables and charts were appropriately used to represent the analyzed data.

3. RESULTS AND DISCUSSION

3.1 Results

Two hundred and nine (209) women whose mean age was 39.6±0.72 years were studied out of which 208 had both cytology and HPV DNA detection done on their specimens. Majority 166 (79.8%) of the respondents were above 30 years of age as only 42 (20.2%) were 30 years and

below. Cytologically, more than half 126 (61.6%) of participants had normal findings but up to 80 (39.0%) had abnormal features while 3 (1.4%) specimens had unsatisfactory smears for cytologic studies. The abnormal cytological features observed were HPV changes 30 (14.4%), HPV changes with inflammation 2 (1.0%), inflammatory changes alone 36 (17.3%), LSIL 3 (1.4%), HSIL 5 (2.4%) and malignant changes 3 (1.4%) (Fig. 1).

Cervical HPV infection based on presence of DNA was detected among 100 (48.1%) of the participants. Table 1 shows the results of HPV DNA testing among women with normal and abnormal cytological findings. Of the 99 participants who had cytology reports and tested positive for HPV, 60 (60.6%) had normal cytologic findings while 39 (39.4%) had abnormal smears.

The mean age of women with cytological HPV changes (34.81±8.96 years) was significantly lower compared to those with other cytological findings (40.40±10.47 years) among the participants (*P* = .005). The mean age of women found to be positive for HPV DNA was only slightly higher; 40.79 (±11.23) years compared to 38.49(±9.47) years among those with no HPV infection. (*P*= .111).

Association between women with HPV infection and those with cytologically detected HPV changes, normal or other abnormal results did not show statistical significance (X^2=3.323, *P* = .344). Almost half 60 (47.6%) of the 127 women with normal cytology were positive for HPV and exactly half 16 (50.0%) of the 32 respondents with cytologic HPV changes were also HPV DNA positive. Among women with cytological inflammatory features alone, 15 (41.7%) of the 36 had HPV infection. Pre-malignant (LSIL and HSIL) and malignant cervical lesions carried the highest percentage of HPV infection as 8 (72.7%) out of 11 were positive for HPV (Table 2).

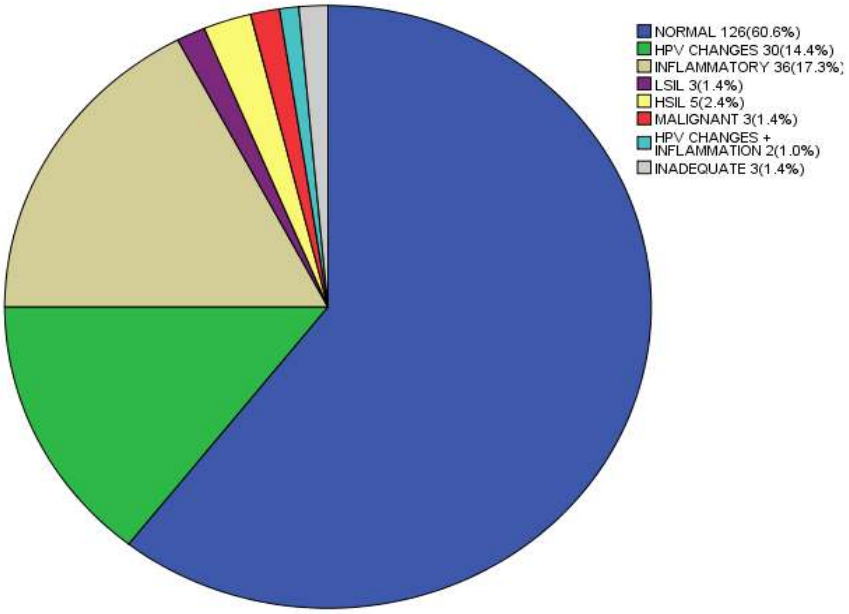

Fig. 1. Cytological findings among the participants (N=208)

Table 1. Results of HPV DNA testing among women with normal and abnormal cytological findings

	HPV DNA positive	HPV DNA negative	Total
Normal cytology	60 (60.6%)	65 (61.3%)	125
Abnormal cytology	39 (39.4%)	41 (38.7%)	80
Total	99	106	205

Tables 3 compared the HPV DNA results based on PCR with cytologically detected HPV changes among the participants. Taking PCR as the gold standard for HPV detection, cytologic HPV changes showed a very low sensitivity of 16.2% but higher specificity of 85.0%. Positive predictive value (PPV) and Negative predictive value (NPV) for cytologically detected HPV changes were found to be 50% and 52% respectively.

3.2 Discussion

Presently, cervical screening for carcinogenic HPV infection is being considered in lieu of cytology even for low-income countries [13]. Negative cytology provides no extra reassurance against cancer beyond that conferred by a negative HPV test result [16]. Also, only about a third of women with HPV infections detectable by DNA testing have recognised cytopathology. Therefore, cytological abnormalities are less sensitive for detection of HPV than molecular testing. Assays for HPV have been introduced to improve the efficiency and maximise the sensitivity of cervical cancer screening. Most importantly, testing negative for carcinogenic HPV provides greater reassurance against cervical pre-cancer/cancer and has greater reproducibility than does cytology-based methods [4]. DNA testing for HPV although substantially more sensitive is somewhat less specific [6] than cervical cytology for the detection of CIN2, CIN3 and cancer. A single negative test for HPV is sufficient to reassure women against cervical cancer over 5 years as they have low risk of CIN3 or cancer during that period regardless of cytological findings [16]. The International Agency for Research on Cancer has endorsed the use of carcinogenic HPV testing alone as an option in primary cervical cancer screening [4]. Testing for HR-HPV is mandatory for women with mild dyskaryosis and for the follow-up of women treated for CIN lesions [17].

Based on efficiency to detect CIN3/cervical cancer and preliminary cost benefit analysis, the combination of a high risk HPV test in conjunction with a cervical smear appears to be the best way of cervical cancer screening [17]. Incorporating HPV testing with cytology could also result in earlier identification of women at high risk of cervical cancer, especially adenocarcinoma (the precursors of which are often missed by cytological methods) and could reduce the incidence of this cancer in women aged 30 years and older [16]. It also predicts increased risk up to ten years later (i.e., a high negative predictive value [18]) and is amenable

Table 2. Association between cervical HPV infection and cytological findings

Cytology	Presence of HPV DNA		Total	X^2	P value
	Positive N (%)	Negative N (%)			
Normal	60 (47.6%)	66 (52.4%)	126 (100%)	4.852	0.678
HPV Changes	15 (50.0%)	15 (50.0%)	30 (100%)		
HPV changes+inflammation	01 (50.0%)	01 (50.0%)	02 (100%)		
Inflammatory	15 (41.7%)	21 (58.3%)	36 (100%)		
LSIL	03 (100%)	00 (0.00%)	03 (100%)		
HSIL	03 (60.0%)	02 (40.0%)	05 (100%)		
Malignant	02 (66.7%)	01 (33.3%)	03 (100%)		

There was no statistically significant association between PCR detected HPV infection and the cytologically detected cervical lesions ($X^2 = 4.852$; P = .678)

Table 3. Comparison of HPV positivity by cervical smear cytology and PCR among the participants

	HPV DNA positive	HPV DNA negative	Total
Cytology positive	16 (TP)	16 (FP)	32
Cytology negative	83 (FN)	90 (TN)	173
Total	99	106	205

(TP: True Positive; TN: True Negative; FP: False Positive; FN: False Negative)
Sensitivity = TP/TP+FN = 16/16+83 = 0.162 = 16.2%
Specificity = TN/TN+FP = 90/16+90 = 0.850 = 85.0%
Positive predictive value (PPV) = TP/TP+FP = 16/16+16 = 0.50 = 50%
Negative predictive value (NPV) = TN/TN+FN = 90/90+83 = 0.52 = 52%

to self-sampling outside the clinic, which allows expanded population coverage [13,11]. The interpretation of the test is objective and does not have the inherent subjectivity of visual screening methods or cervical cytologic assessment [6].

Different reports have highlighted the supplementary role of HPV DNA testing in primary/secondary cervical cancer screening with more promising outcomes [19,20]. The utility/acceptability of HPV DNA testing in cervical cancer screening has shown better prospects with introduction of self sampling techniques and use of other body fluids (e.g. urine) in detecting the virus [21,22]. This may obviate several hindrances to cervical cancer screening that may be due to socio-cultural and/or religious issues reducing the acceptability of current conventional screening method. It has recently been posited that cervical cancer screening using HPV DNA testing might overtake conventional cytology and cost countries less money while providing greater safety [23,24].

Several studies from Nigeria had reported varying prevalences of cervical HPV infection among different population groups. However very scanty literature is available to compare the presence of cervical HPV infection with corresponding cytopathological features especially in Northern Nigeria where cervical cancer is more prevalent.

The age group of participants from this study with majority being above 30 years of age makes them suitable for the use of HPV DNA testing as an appropriate tool for cervical cancer screening. Thus majority of those with positive HPV DNA test from this study may be harbouring a persistent infection which is the major risk factor for the development of cervical cancer [2].

Out of 99 participants who were HPV DNA positive and also had cytology reports, more than half (60.6%) revealed normal cytological findings while 39 (39.4%) showed abnormal features cytologically. This means that up to 21.2% (21) of women with confirmed HPV infection did not show any cytologic abnormality. This conforms to some findings that reported up to 15% to 35% of women with CIN 3 or even cancer being missed by cervical cytology [14]. In another study, 15.1% of women above 30 years of age with persistent hr-HPV infection were preceded by normal cytology [25]. This makes imperative the need for additional/alternative screening method for cervical cancer aside cytology. Combination of hr-HPV testing and cytology provides the best option for screening even in low resource settings as northern Nigeria [26]. Additionally, incorporation of HPV testing in cervical cancer screening will even lead to better detection of particularly adenocarcinoma whose precursors are often missed by cytological methods [16].

A high prevalence (72.7%) of HPV infection was observed among women with either premalignant (LSIL or HSIL) or malignant cervical lesions. Persistent HPV infection is a well known precursor of both premalignant and malignant cervical lesions. A study in France reported up to 82% HPV positivity among women with CIN 2/3 [27]. Almost half (41.7%) of the women with cervical inflammation without HPV changes observed in this study were HPV DNA positive. Infections including HPV are a common cause of cervical inflammation. Cervical inflammation has been found to be likely associated with high-grade lesions with a possibility of being a cofactor for high-grade cervical lesions in women infected with oncogenic HPV [28].

We observed that only 50% of women with PCR confirmed HPV infection were found to have corresponding cytologic changes suggestive of the infection. This may not be unconnected with the fact that clearance of cervical HPV infection precedes cytologic regression and only about a third of women with HPV infection have recognised cytopathology [4,29]. This may be supported by the relative low sensitivity (16.2%) and higher specificity (85.0%) of cervical smear cytology in detecting cervical HPV infection compared to PCR (the gold standard) which is also not unusual [6,4]. The positive predictive value (PPV) of 50% and negative predictive value (NPV) of 52% further reaffirms that barely half of all women with or without HPV infection were picked or ruled out by cervical cytology. It has portrayed the poor performance of cervical cytology in detecting HPV infection while further justifying the need to add HPV detection in primary cervical cancer screening especially for women above 30 years of age in whom persistence of the infection may eventually translate in cervical cancer.

Granted that we have not carried out colposcopy or histology on the participants as such could not confirm the presence of cervical lesions, HPV testing in conjunction with cytology has been reported to improve the screening efficacy of cytology alone while allowing for a more effective and safe primary screening program with increased screening intervals [25,30].

4. CONCLUSION

In conclusion, the fact that combining cytology and hr-HPV testing is not likely to miss any clinically relevant cervical lesion in addition to offering longer screening intervals makes it the best cervical cancer screening option even in developing countries. With the recent additions such as the use of other specimens like urine, swabs for cervical self-sampling and the automation of HPV testing methods that require little or no training even by unskilled personnel, the future of HPV testing holds promise in cervical cancer screening.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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