Diagnostic Value of Conventional Cytology in Detecting Human Papilomavirus in Cervical Smears

[1]IK Auwal, [2]AT Atanda, [3]M Amin, [3]EE Ella and [4]J Tukur

[1]Department of Medical Microbiology and Parasitology, Faculty of Medicine, Bayero University, Kano, Nigeria
[2]Department of Pathology, Faculty of Medicine, Bayero University, Kano, Nigeria
[3]Department of Medical Microbiology, Ahmadu Bello University, Zaria, Nigeria
[4]Department of Obstettrics & Gynaecology, Faculty of Medicine, Bayero University, Kano, Nigeria

Abstract

***Background**: For a country like Nigeria where conventional Pap smear is still the mainstay of screening for preneoplastic lesions of cervical cancer there is need to determine the diagnostic value of this technique for detecting HPV, the most important aetiological agent of this cancer.*

***Materials and Methods**: 300 women were subjected to conventional Pap smear with simultaneous sampling for E6 gene of HPV 16 and 18 by PCR, and the sensitivity, specificity and positive predictive value of Pap smear for detecting HPV infection (Low Grade Intraepithelial Lesions) determined.*

***Results**: Conventional Pap smear showed a sensitivity of 34.3%, specificity of 99.3% and positive predictive value of 85.7% ($p < 0.05$).*

***Conclusion**: This study concludes that there is need for adoption of more sensitive methods such as liquid-based cytology and HPV testing for detecting early changes such as HPV infection in cervical epithelium in view of the low sensitivity of conventional Pap smear. This will help in better triaging of patients for follow-up.*

Keywords: *Human Papillomavirus; Polymerase Chain reaction; Sensitivity; Pap smear*

Introduction

Human Papilloma Virus (HPV), implicated as the most important aetiopathogenetic agent in the causation of pre-neoplastic and neoplastic lesions of the cervix, has a variable prevalence worldwide. Prevalence surveys in women 15 to 65 years of age locally in Nigeria and internationally have been shown to vary from 26.3% in Ibadan, Nigeria, 33.2% in Benin, West Africa, 66.1% in Burkina Faso and to as low as 2% in Hanoi, north Vietnam.[1-5]

The HPV serotypes have been classified into oncogenic and non-oncogenic serotypes. Over

Correspondence to: Dr Akinfenwa Taoheed Atanda, Department of Histopathology, Aminu Kano Teaching Hospital, Kano, PMB 3452, Kano E-mail address: akinzo123@gmail.com

No conflicts of interest have been declared by the authors

a hundred serotypes are currently recognized; 40 of which infect the genital tract.[6] The oncogenic serotypes, particularly 16 and 18, have been shown to be responsible for most of the progression to cancer.[7, 8] Detection of the oncogenic serotypes is therefore central to prevention of cervical cancer which is the second most common cancer in women worldwide and the predominant cancer in women in Kano.[9, 10]

Thus, accurate diagnosis of HPV infection is required, particularly in Nigeria where screening is still an opportunistic event for most women. The most routinely and widely employed screening method in Nigeria still remains the conventional cervical smear (Papanicolaou smear or Pap smear) for detecting not only the cytopathic effects of HPV infection but also other progressive atypical changes. Therefore it is essential to determine the diagnostic value of this method of HPV detection. The aim of this study was to determine the diagnostic value of conventional pap smear as compared with Polymerase Chain Reaction (PCR) in detecting HPV infection in cervical scrapings.

Materials and Method

This was a multicentred descriptive cross-sectional study conducted in two referral hospitals in Kano, Aminu Kano teaching Hospital (AKTH) and Murtala Muhammad Specialist Hospital (MMSH), Kano. Sample size of approximately 300 was determined using the method described by Sar Mukaddam and Garad[11] with prevalence of 26.3.[1]

Three hundred women from the ages of 18 years and older who attended gynaecology clinics in AKTH and MMSH during the study period were randomly recruited and having obtained their signed or thumb-printed consent were subjected to conventional pap smear and also had samples taken for PCR. Pregnant women, women who have had hysterectomy or cervical conization, or who are physically or mentally challenged as well as women bleeding per vaginam were excluded. Cases diagnosed as high grade lesions or cervical cancers on cytology were also excluded as these do not typically show HPV cytopathic changes.

Sterile wooden Ayres' spatula was used to collect exfoliated cells from the ectocervix and endocervix, smeared on clean labelled glass slide and immediately fixed in 95% ethanol. These were stained by Papanicolaou technique and reported on daily basis at the histopathology unit of AKTH. At the point of collection a second sample was also collected and immersed in 5mls of phosphate buffered saline (a transport medium) in universal rubber container, stored at 4°c and transported in ice packs to the biotechnology and research center, Ahmadu Bello University, (ABU) Zaria for viral DNA detection using PCR.

DNA extraction was carried out using the ZR genomic DNA™ –tissue mini prep kit (Zymoresearch cooperation, South Africa). E6 gene of HPV 16 and 18 viral DNA amplification was carried out using the Primer set (Inqaba biotechnical industries Pty Ltd, South Africa). The reaction was performed according to the PCR manufacturer's protocol (Fermentas life science, South Africa). The PCR procedure was validated with known amplicons of HPV 16 and 18 DNA and two of our biopsy proven but excluded malignant cases.

Cytological criteria employed for the diagnosis of HPV changes [Low Grade Squamous Intra-epithelial Lesion (LSIL) of Bethesda classification][12] were epithelial cells with

- well defined, optically clear perinuclear halo
- dense peripheral rim of cytoplasm and
- nuclear abnormalities (hyperchromasia, nuclear membrane wrinkling and multinucleation) with nuclei size similar to superficial or intermediate cells
- mature cytoplasm

Amplified HPV DNA from PCR was digitally monitored (as shown in Figure 1) and ensuing data analyzed using SPSS version-10 statistical package. The sensitivity, specificity and positive predictive value were calculated using the

formulae TP/TP+FN, TN/TN+FP and TP/TP+FP respectively; (TP: True Positive; TN: True Negative; FP: False Positive and FN: False Negative).

Results

Of the 300 women recruited in this survey, 35 were positive for HPV DNA by PCR, giving a prevalence of 11.7 (8.3 – 15.9 at 95% Confidence level). These included 3 (8.6%) of the subjects positive for HPV 16 only, 6 (17.1%) positive for HPV 18 only and co-infection in 26 (74.3% of the women. Sixteen (45.7%) of the women were 30 years of age and younger while the remaining 19 (54.3%) women were older than 30 years of age. Of the 35 cases positive by PCR for HPV DNA, cytology, employing the study criteria for HPV changes was able to correctly detect 12 of the cases, as shown in Table 1. The remaining 23 cases had been diagnosed as negative by Pap smear. This thus gives a sensitivity of 34.3%, specificity of 99.3% and positive predictive value of 85.7% (p-value: 0.000; < 0.05).

Table 1: Shows comparison of HPV possitivity by cervical smear cytology and PCR among the 300 women

	PCR Positive	PCR Negative	Total
Pap Positive	12 (TP)	2 (FP)	14
Pap Negative	23 (FN)	263 (TN)	286
Total	35	265	300

(TP: True Positive; TN: True Negative; FP: False Positive and FN: False Negative).

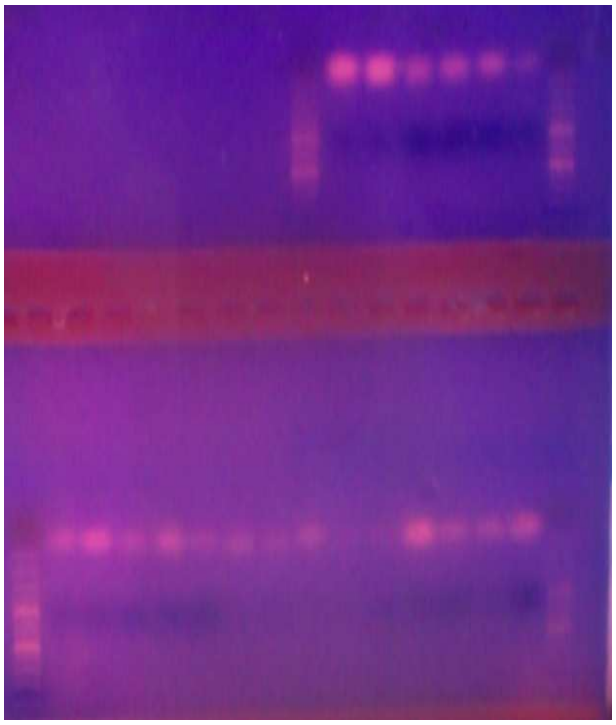

Fig.1: shows digitally monitored amplicons on Agarose gel electrophoresis stained with ethidium bromide

Discussion

The conventional Pap smear has shown great resilience in the face of increasing criticisms. These criticisms have stemmed not only from inherent limitations in obtaining representative samples but also from high false negative rates reported for low grade lesions.[13, 14] This study's finding of sensitivity of 34.3% for detecting HPV changes (LSIL) by conventional pap smear buttresses conclusions drawn from systematic review of the most unbiased estimates which have found sensitivity ranging between 30% and 87%.[15]

The low sensitivity of conventional pap smear for detecting HPV changes may stem from the biology of the virus. In the latent (unexpressed) phase of infection viral load per cell is low and this stage cannot be detected by cytology or visual inspection.[16] Studies have shown that cytological abnormalities are more frequent in those with high viral loads.[17, 18] In addition to this, there is positive correlation with increase in viral load from low to high grade lesions for infection with HPV 16[19] while HPV 18, which was seen in 32 (91.4%) of our subjects, more frequently fails to impart readily detectable cytological abnormalities.[20]

Failure of cytology to detect HPV infection may also stem from its latency phase. In this phase

the virus is dormant and may remain so for several months to years[21] depending on the interplay between the immune system of the host and the virus, thus if the host is screened within this phase, pap smear may be negative. This is reflected in our findings where a significant number (45.7%) of women positive for HPV DNA were under 30 years of age and more likely harbour recent infections.

The method of collecting cervical smear samples with the conventional spatula, as was done in our study, being the predominant method of collection in most centres in the country, also has a negative impact on smear cytology. Hutchinson et al[22] have shown that only about 20% of cells collected by the Ayre's spatula are actually transferred to the glass slide, the remaining being discarded with the spatula. In this scenario, a large number of representative cells may thus be lost. Further confounding factors related to this method of collection is the observation that has been made about persistence in the expression of E-cadherin by dysplastic cells beyond the basal layer. Such persistent expression results in down-regulation of normal exfoliation of superficial cells and has been correlated with false negative smears.[23]

Inter-observer errors or variation is another important cause of false negative smears. Even though the involvement of more than one individual at different levels of slide review was done in this study as a way to reduce error rates, such review errors have been found to be important causes of false negative smears.[24]
From the foregoing, while sensitivity of conventional pap smear in the detection of low grade lesions has been shown to be low, a high specificity of 99.3% was found in our study and this is consistent with studies that have documented specificity ranging between 97% and 100%.[13, 14] We also found a high positive predictive value of 85.7% which is comparable to the 87.8% documented by another study.[25] These simply imply that the conventional Pap smear gives a high degree of certainty when it renders a diagnosis of positive for HPV infection but diagnostically weak because of high false negativity.

In conclusion our study has shown that even though detection of HPV changes in cervical smears by conventional pap smear has high specificity and positive predictive value, its low sensitivity for detecting these low grade lesions necessitates its substitution with more sensitive techniques; and based on literature review, liquid based cytology, which has a higher sensitivity than the conventional Pap smear, and HPV testing appear to be better alternatives for triaging of early preneoplastic lesions of the cervix, particularly among women 30 years and older.[26, 27]

Study Limitations

- Due to cost limitations PCR was done to identify only oncogenic serotypes HPV 16 and 18,
- Obtained samples were hospital-based and even though the women came from all parts of Kano, findings may underestimate the true HPV pattern.

References

1. Thomas JO, Herrero R, Omigbodun AA, *et al.* Prevalence of papillomavirus infection in women in Ibadan, Nigeria: A population-based study. Br J Cancer 2004; 90(3): 638-645.
2. Piras F, Piga M, De Montis A, *et al.* Prevalence of Human papaillomavirus infection in women in Benin, West Africa. Virol J. 2011; 8: 514
3. Didelot-Rousseau MN, Nagot N, Costes-Martineau V, *et al.* Human Papillomavirus genotype distribution and cervical squamous intra-epithelial lesions among high-risk women with and without HIV-1 infection in Burkina Faso. Br J Cancer. 2006; 95: 355-362.
4. Molano M, Posso H, Weiderpass E, *et al.* Prevalence and determinants of HPV infection among Colombian women with normal cytology. Br J Cancer. 2002; 87: 324-333.

5. Anh PTH, Hieu NT, Herrero R, *et al.* Human papillomavirus infection among women in South and North Vietnam. Int J Cancer. 2003; 104: 213-220.
6. de Villiers EM, Fauquet C, Broker TR, Bernard HU and zur Hausen H. Classification of Papillomaviruses. Virology 200; 324: 17-27.
7. Zur Hussen H. Papillomaviruses in the causation of human cancers – a brief historical account. Virology. 2009; 384: 260-265.
8. Bosch FX, Munos MM, Munos N, *et al.* Prevalence of Human papillomavirus in cervical cancer: a worldwide perspective. International Biological study on Cancer (IBSCC) study group. J Natl Cancer Inst; 1995; 87: 796-802.
9. Parkin DM and Bray F. The burden of HPV-related cancers. Vaccine 2006; 24(suppl 3): S11-S25.
10. Mohammed AZ, Edino ST, Ochicha O, Gwarzo AK and Samaila AA. Cancer in Nigeria: a 10-year analysis of the Kano cancer registry. Niger J Med. 2008 Jul-Aug;17(3):280-284.
11. Sar Mukaddam SB and Garad SG. Validity of Assumption while determining sample size. Indian J Comm Med. 2006; 29(2): 2004-2006.
12. Solomon D, Davey D, Jurman R, *et al.* The 2001 Bethesda System Terminology for reporting results of cervical cytology. JAMA 2002; 287: 2114-2119.
13. Koss LG. Cervical (pap) smear. New directions. Cancer 1993; 71(suppl 4): 1406-1412
14. Soost HJ, Lange HJ, Lehmacher W and Ruffing-Lullman B. The validation of cervical cytology: sensitivity, specificity and predictive values. Acta Cytol 1991; 35: 8-14
15. Nanda K, McCrory DC, Myers ER, *et al.* Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Ann Intern Med. 2000; 132(10): 810-819.
16. Natural History of HPV. Available at http://www.asccp.org/practicemanagement/hpv/naturalhistoryofhpv/tabid/5962/default.aspx. Accessed February 15, 2012.
17. Lillo FB, Lodini S, Ferrari D, *et al.* Determination of human papillomavirus (HPV) load and type in high-grade cervical lesions surgically resected from HIV-infected women during follow-up for HPV infection. Clin Infect Dis. 2005; 40: 451-457.
18. Nindl I, Greike C, Zahm DM, Stockfleth E, Hover H and Schneider A. Human papillomavirus distribution in cervical tissues of different morphology as determined by hybrid capture assay and PCR. Int J Gynecol. 1997; 16: 197-204.
19. Swan DC, Tucker RA, Tortolero-Luna G, *et al.* Human Papillomavirus (HPV) DNA copy number is dependent on grade of cervical disease and HPV type. J Clin Micrbiol. 1999; 37: 1030-1034.
20. Woodman CB, Collins S, Rollason TP, *et al.* Human Papillomavirus type 18 and rapidly progressing cervical intraepithelial neoplasia. Lancet 2003; 361: 40-43.
21. Watson RA. Human Papillomavirus: Confronting the Epidemic – A Urologist's Perspective. Rev Urol. 2005; 7(3): 135-144.
22. Hutchinson ML, Agarwal P, Denault T, Berger B and Cibas ES. A new look at cervical cytology. ThinPrep multicenter trial results. Acta Cytol. 1992; 36: 400-504.
23. Felix JC, Lonky NM, Tamura K, *et al.* Aberrant expression of E-cadherin in cervical intra-epithelial neoplasia correlates with a false-negative Papanicolaou smear. Am J Obstet Gynecol 2002; 186 (6): 1308-1314.
24. Pairwuti S. False-negative Papanicolaou smears from women with cancerous

and precancerous lesions of the cervix. Acta Cytol 1991; 35(1): 40-46
25. Ferris DG, Heidemann NL, Litaker MS, Crosby JH and McFee MS. The Eficacy of Liquid-Based Cervical Cytology Using Direct-to-Vial Sample Collection. J Fam Pract 2000; 49: 1005-1011.
26. Sherwani RK, Khan T, Akhtar K, *et al.* Conventional Pap smear and liquid based cytology for cervical cancer screening – a comparative study. J Cytol 2007; 24: 167-172.
27. Goldie SJ, Kim JJ and Wright TC. Cost-effectiveness of human papillomavirus DNA testing for cervical cancer screening in women aged 30 years or more. Obstet Gynecol 2004; 103(4): 619-631.