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ORIGINAL ARTICLE

Molecular detection of human papillomavirus from abnormal cervical cytology of women attending a tertiary health facility in Ido-ekiti, southwest Nigeria

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Keywords

Cervical cytology • HPV DNA • *women*

Summary

Background. *Human papillomavirus (HPV) has been implicated as one of the principal causes of cervical cancer, which is the second highest cause of cancer deaths among Nigerian women.*
Objective. *This study was aimed at determining the presence of HPV DNA in abnormal cervical cytology of a group of women who were screened using Papanicolaou staining technique.*
Methodology. *A total of 200 women attending the Obstetrics and Gynaecology clinic of the Federal Teaching Hospital, Ido-Ekiti, were screened by means of conventional Pap smear screening, while positive samples underwent molecular analyses by means of DNA isolation techniques and polymerase chain reaction (PCR).*
Result. *Results revealed that 14 (7%) of the subjects were positive for abnormal cytology. Abnormalities found among the subjects included: low-grade squamous intraepithelial lesions (LSIL), which constituted 50% of the total abnormal smears, high-grade squamous intraepithelial lesion (HSIL) and atypical squamous cells of undetermined significance (ASCUS), which were 28.6% and 21.4%, respectively. Molecular analyses showed that all the samples from abnormal cervical cytology subjected to HPV DNA extraction and gene amplification contained HPV DNA.*
Conclusions. *The high prevalence of HPV DNA in abnormal cytology gives credence to the fact that the presence of HPV is a critical indicator of the development of cervical cancer. Thus more effort should be put into vaccine production and distribution in order to reduce the incidence of cervical cancer in Nigeria.*

Introduction

Human papillomavirus (HPV) belongs to the family Papillomaviridae, which is capable of infecting humans. Like all papillomaviruses, HPV establishes productive infection only in keratinocytes of the skin or mucous membranes [1]. HPV is a small non-enveloped icosahedral DNA virus that replicates in the nucleus of squamous epithelial cells [2]. High-risk human papillomaviruses (HR-HPVs) are able to infect several types of epithelial cells, but more frequently cause cancer in the uterine cervix [3].
Various risk factors are associated with the occurrence of HPV infection; these include: smoking, multiparity, early onset of sexual intercourse, oral contraceptive use and Human Immunodeficiency Virus. They contribute greatly to an individual's chances of developing cervical cancer [4]. Cervical cancer ranks as the second most frequent cancer among women in Nigeria, and the most frequent cancer among women between 15 and 44 years of age. About 23.7% of women in the general population are estimated to harbor cervical HPV infection at any given time [1]. The prevalence of HPV genotypes in cervical cytological samples varies greatly in different geographical regions and shows a strong correlation with cervical cancer incidence. Epidemiological studies have consistently shown that the most important determinants of HPV infection in women are the number of sexual partners, the age of initiation of sexual activity, and the sexual behaviour of the male partner [5].
Clifford *et al.* randomly selected women from 11 countries for HPV DNA testing, using GP5+/6+ PCR analyses [6]. Women aged 15-74 years who did not have cytological abnormalities were included (n = 15613). They found that age-standardized prevalence varied nearly 20-fold among different populations: from 1.4% in Spain, to 25.6% in Nigeria. Overall, age-standardized HPV prevalence was five times higher in sub-Saharan Africa than in Europe, with intermediate prevalence in South America and Asia. In terms of types, HPV type 16 was the commonest type in all regions except sub-Saharan Africa, where HPV type 35 was equally common.

Materials and methods

Ido-Ekiti is located in Ido/Osi Local Government Area of Ekiti State, Nigeria. It is situated in the Northern part of the state, where the routes from Oyo, Osun and Kwara states converge. Ido-Ekiti is the headquarters of Ido/Osi local council. The hospital is located in Ido-Osi Local Government Area of Ekiti State, which lies south of

Kwara and Kogi State, East of Osun State and north of Ondo State, and has a total land area of 5887.890 sq km. The 2006 population census by the National Population Commission put the population of Ekiti State at 2,384.212 people.

Study population

The study was carried out among women attending the Gynaecology Clinic of the Federal Teaching Hospital, Ido-Ekiti who were between the ages of 15-64 years, willing to participate and met the inclusion criteria. A structured close-ended questionnaire was administered to these patients after informed consent had been obtained, followed by clinical examination.

Inclusion criteria

Female patients attending the Obstetrics and Gynaecological unit, between 15 to 64 years of age, who consented to take part in the study, and non-patients who met other inclusion criteria and agreed to participate in the study.

Exclusion criteria

Female patients over 65 years or under 15 years; male patients; patients who do not give consent to take part in the study and patients who have had total abdominal hysterectomy.

Ethical clearance

Approval for this study was obtained from the Ethics Review Committee of the Federal Teaching Hospital, Ido-Ekiti, after which informed consent was obtained from patients and/or parents and guardians. The study was done at no financial cost to the subjects, and information from the patients and/or parents and guardians were confidential.

Sample collection and analysis

A structured close-ended questionnaire was administered to the subjects after due consent had been obtained; cervical smears were collected by a gynaecologist after visual inspection. The smears collected were immediately fixed to slides before being transferred to the laboratory for processing. The fixed smears were stained by the cytotechnologist using the Papanicolaou staining procedure and read by a histopathologist using a light microscope. The smears were classified as normal, inflammatory, abnormal (epithelial lesion) or unsatisfactory.

Data Analysis

Descriptive statistics (mean, frequency, standard deviation and percentage) and a graph were used to present the results, in order to provide a clear representation of the data analyzed. The statistical package for social science version 17.0 for windows was used to test for the level of significance of the results obtained. Both continuous and discrete variables were generated. The relationship between categorical variables and outcome of interest was tested by means of Chi-square test at 95% ($p < 0.05$) confidence interval.

PCR/DNA extraction

Molecular analyses began with the extraction of DNA from the cervical smears that were positive for intraepithelial neoplasia. The coverslips present on the slides during Pap staining were removed with the aid of xylene, and the smears carefully removed by means of sterile scapel blades. A DNA extraction kit produced by Integrated DNA Technologies, USA, was utilized for DNA extraction. The extracted smears were transferred to 1.5ml tubes for DNA extraction. Buffy coats and proteinase K were used to lyse the cells of the virus, and DNA extraction was done by means of chloroform. PCR components were produced by Integrated DNA technologies (USA); the PCR reaction contained primers Gp5: 5'TTTGTTACTGTGGTAGATACTAC-3'and Gp6: 5'GAAAAATAAACTGTAAATCATATTC-3'. The reaction was programmed as follows. Initial denaturing step at 95°C for 15 min, 10 cycles of 30s at 94°C, 90s at 72°C, followed by 30 cycles of 30s at 94°C, 90s at 63°C and 90s at 72°C, with a final extension of 72°C for 10 min. A DNA band of 150 kb was considered a positive result.

Gel electrophoresis procedure

Agarose (3g) was heated in solution in a microwave until it had completely dissolved, and was then allowed to cool in a water bath set at 50-55°C. The required numbers of combs were placed in the gel tray; 5 ul of ethidium bromide was added to the cooled gel, which was then poured into the gel tray. The gel was allowed to cool for 15-30 min at room temperature. The combs were removed and placed in an electrophoresis chamber and covered with buffer (TAE). DNA and standard (ladder) was loaded onto the gel, which was electrophoresed for at least an hour. DNA bands were visualized by means of a gel imaging system.

Results

Table I shows the socio-demographic and reproductive characteristics of the respondents; about two-thirds 133 (66.5%) of the women had attained their sexual debut at ≤ 15 years of age. Married women accounted for 176 (88.0%), and 146 (73%) had a tertiary education.

Figure 1 shows the distribution of the abnormal cervical cytology by type of abnormality. Three different types of abnormality were found among the subjects. Low-grade squamous intraepithelial lesions (LSIL) constituted the main form of abnormal cytology, accounting for 50% of the total abnormal smears. High-grade squamous intraepithelial lesions (HSIL) were 28.6%, while atypical squamous cells of undetermined significance (ASCUS) were 21.4%.

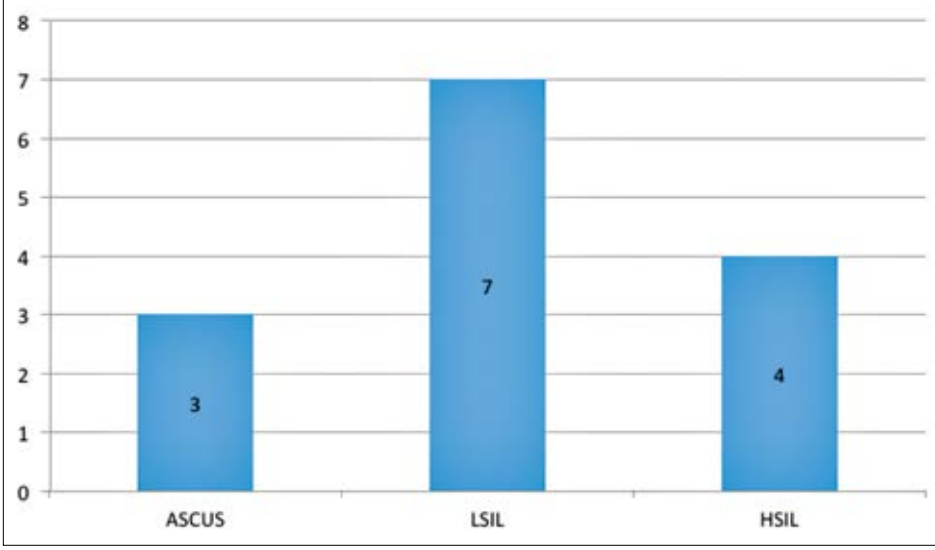
All the samples presenting abnormal cervical cytology underwent HPV DNA extraction and gene amplification; all contained HPV DNA (Fig. 2). Fourteen abnormal

Tab. I. Socio-demographic and reproductive characteristics of the respondents.

Variables	Frequency (%)	Variables	Frequency (%) N = 200
Age (years)		**Age at sexual debut (years)**	
15-24	5 (2.5)	≤ 15	133 (66.5)
25-34	50 (25.0)	> 15	67 (33.5)
35-44	80 (40.0)		
45-54	47 (23.5)	**Parity**	
55-64	18 (9.0)	0	45 (22.5)
		1-3	116 (58.0)
		> 3	39 (19.5)
Level of education			
Primary	4 (2.0)	**Previous genital infection**	
Secondary	23 (11.5)	Yes	126 (73.0)
Tertiary	146 (73.0)	No	74 (37.0)
None	27 (13.5)		
Marital status		**Number of sexual partners**	
Single	19 (9.5)	0-1	174 (87.0)
Married	176 (88.0)	> 1	26 (13.0)
Divorce	5 (2.5)		
Alcohol intake		**Male partner circumcision**	
Yes	31 (15.5)	Yes	192 (96.0)
No	169 (84.5)	No	8 (4.0)
Tobacco use			
Yes	9 (4.5)		
No	191 (95.5)		
Type of marriage**			
Monogamy	57 (31.5)		
Polygamy	124 (68.5)		
Use of oral contraceptives			
Yes	111 (55.5)		
No	89 (44.5)		

** N=181

Fig. 1. Distribution of abnormal cervical cytology types amongst subjects in relation to acquisition of HPV infection.

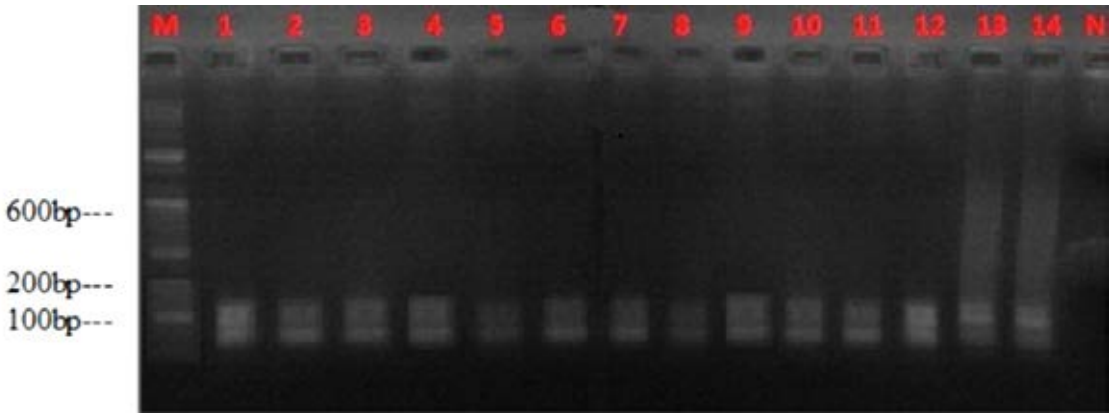


ASCUS: Atypical cells of uncertain significance

LSIL: Low-grade squamous intraepithelial lesion

HSIL: High-grade squamous intraepithelial lesion

Fig. 2. Positive PCR bands with corresponding HPV genotypes (1kb plus DNA ladder, 0.9% agarose gel stained with ethidium bromide).



Lane 1 represents (M) High ranger 1kb DNA Ladder: lanes 2-15 are amplified HPV genome: 1,2,3,4,5,6,7,8,9,10,11,12,13,14; lane 16 represents negative control (NC). Left hand side: molecular size of marker bands.

cervical cytology samples underwent molecular studies. Of these 14 samples, 7 had low-grade squamous intraepithelial lesions, 4 were high-grade squamous intraepithelial lesions and the remaining 3 atypical squamous cells of uncertain significance. The molecular results showed the analysis of HPV DNA in cervical samples to be a predictor of seropositivity. The 1-14 bands indicated in the gel electrophoresis image (1-14) are indicative of the presence of HPV DNA. Lane 1 represents (M) High ranger 1kb DNA Ladder; lanes 2-15 are amplified HPV genome: 1,2,3,4,5,6,7,8,9,10,11,12,13,14, and lane 16 shows a negative control (NC).

Discussion

Results showed that the largest number of abnormal smears were of low-grade squamous cell carcinoma (LSIL), which accounted for about 50% of abnormalities. This is similar to the results obtained by Obaseki and Nwafor (2013), who reported that LSIL accounted for the highest percentage (66.27%) of abnormalities found [7].

In this study, HPV DNA was extracted from all the samples with abnormal cytology; all of the subjects with abnormal cervical cytology were also positive for the presence of HPV DNA. This is in line with the results of the study by Sharifah et al. (2009), in which all 38 abnormal cervical cytology smears analysed were positive for the presence of HPV DNA [8]. HPV DNA was discovered in all the cases of low-grade quamous cell carcinoma, high-grade squamous cell carcinoma and atypical squamous cells of uncertain significance. This high prevalence of HPV DNA in abnormal cytology observed in this study also lends credence to several studies which have cast doubt on the existence of HPV DNA-negative abnormal cervical smears [8]. Indeed, various studies have shown a high prevalence of HPV DNA in abnormal cytology; this is in agreement with the presence of HPV DNA in all our abnormal cervical smears [4]. The frequency of HPV in abnormal cervical cytology also underlines the strong correlation between cervical cancer, HPV DNA positivity and abnormal cervical cytology.

Even though genotyping could not be done in this study, the presence of HPV DNA in all abnormal cervical smears reveals a dire need for a national program for vaccination against HPV infection in Nigeria. There is also a need to look at the various screening methods utilized for detecting HPV infection and, where possible, molecular techniques should be incorporated alongside with serology and the Papanicolaou test.

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Authors' contributions

OMK and KTO conceived and designed the research. KAD and KTO co-ordinated the data and Pap smear collection on the field. OMK, KTO and KAD performed the data quality control and optimized the informatics data base. KAD and KTO performed the statistical data analyses. KTO, OMK and KAD evaluated the results. KAD, KTO and OMK wrote the manuscript. All authors revised the manuscript and gave their contribution to improve the paper. All the authors read and approved the final manuscript.

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