Original Article

Value of high-risk human papillomavirus 16 deoxyribonucleic acid testing with cytological entities in peri and postmenopausal women

ABSTRACT

Background: Genital human papillomavirus (HPV) infection is a sexually transmitted disease that is caused by HPV. Some types of HPV, called high-risk (HR) types may cause cell changes that sometimes lead to cervical cancer. HPV screening has been proposed for symptomatic female population; however, Pap test is the main stay in low resource setting.

Aim: To detect HR HPV 16 positivity in perimenopausal and postmenopausal women and its association with cytological entities diagnosed on Pap smear.

Materials and Methods: Pap smears and cervical scrapes were collected from 230 women consisting of 120 perimenopausal women approaching menopause and 110 postmenopausal women with a cervix after cessation of menstruation and processed as per routine procedure for detection of HR-HPV 16 deoxyribonucleic acid (DNA). Cytologically abnormal HPV 16 negative cases were also tested for other HR-HPV types.

Results: Among the perimenopausal women 12 (10%) cases were positive for HR-HPV 16 consisting of 6 (5%) abnormal cases and 108 (90%) were HPV 16 negative consisting of 5 (4.1%) abnormal cases. However, among 110 postmenopausal women 14 (12.7%) were positive for HPV 16 DNA consisting of 6 (5.4%) abnormal cases and 96 (87.2%) were HPV 16 negative consisting of 4 (3.6%) abnormal cases. HPV 16 negative abnormal cases (9) were positive for low risk-HPV 6/11 consisting of atypical squamous cells (3) and low-grade squamous intraepithelial lesions-HPV (6).

Conclusions: There is not much variation in HPV 16 positive cases in peri and postmenopausal women. By combining HPV DNA testing with Pap smear more cases having potential for pre-cancer lesions may be detected; however, HPV test cannot replace the Pap smear in low resource setting.

Key words: Human papillomavirus 16 deoxyribonucleic acid; menopause; Pap smear; uterine cervix.

Introduction

Human papillomavirus (HPV) infections are known to be sexually transmitted among heterosexual couples. Some types of HPV, called high-risk (HR) types may cause cell changes that sometimes lead to cervical cancer. The relation between the cervical cancer and HPV has been well-established, but it is also true that majority of women infected with HPV will not develop lesions. HPV infection are transient in young sexually active women, but its persistence may lead to causing cervical cancer in older women.[1-5] HPV deoxyribonucleic acid (DNA) testing has also been proposed as routine screening method for the sexually active female population,[6,7] but in limited resource setting HPV screening is not cost-effective than Pap smears. Because HPV does not always cause symptoms and women are unaware that they are infected with HPV, Pap test detects abnormal cell changes and HPV DNA test detects the virus that causes the abnormal cell changes so HPV

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DNA testing is more effective than cytology in preventing invasive cervical cancer, which showed a high frequency (98%) of HPV in India[8] as compared to those reported from other parts of the world and HR HPV 16 is the type exclusively (80-90%) prevalent in cervical cancer. There is a lot of literature on HPV DNA testing of young women, but little attention has been paid to mature women aged 45 years or older and they are not usually tested by HPV DNA test because of approaching menopause while Pap smears is recommended for older women and is the main stay of cervical cancer screening in a low resource settings.[9] The present study has been undertaken to evaluate HPV 16 DNA positivity in association with cytological entities on Pap smears of perimenopausal and postmenopausal women.

Materials and Methods

Patient population

During the routine cervical cancer screening program Pap smears and cervical scrapes from 230 women, belonging to perimenopausal (120) and postmenopausal (110) status, were collected. All postmenopausal women had cervix and they were not taking hormone replacement therapy. The age of perimenopausal women ranged from 46 years to 52 years with a mean age of 49 years (±1.64) and age of postmenopausal women, after cessation of menstruation, ranged from 53 years to 58 years with a mean age of 55.2 (±1.51). The Pap smears were collected by Ayre's spatula along with the details regarding age, parity, clinical symptoms and clinical diagnosis on a predesigned proforma from LN Hospital of Delhi. Initially, the cervical scrapes were collected by Ayre's spatula from the cervix to make a smear on a glass slide and fixing the smear in ethanol and then the tip of the spatula with residual samples were broken and kept in the bottle containing phosphate buffered saline buffer and then frozen until tested for DNA extraction and further for polymerase chain reaction (PCR) DNA sequencing for HR HPV 16. Pap smears were stained by Papanicolaou stain and The Bethesda System[10] was used for categorization of Pap smears. The Pap smears results were classified based on Bethesda system as Negative for intraepithelial lesion or malignancy (NILM) or epithelial cell abnormalities. The abnormalities were further classified as atypical squamous cells (ASC), low-grade squamous intraepithelial lesions (LSIL) encompassing HPV/mild dysplasia/cervical intraepithelial neoplasia I, high-grade squamous intraepithelial lesions (HSIL) and invasive cancer.[11] These abnormal cases were investigated by colposcopy and guided biopsies were carried out wherever required and further managed by the gynecologist.

DNA extraction and HPV PCR

Genomic DNA was isolated from cervical samples by using standard proteinase K digestion followed by phenol-chloroform extraction procedures routinely performed in the laboratory. The quality and quantity of DNA were measured either on an ethidium bromide-stained 1% agarose gel using Hind III-digested lamda marker or by standard spectrophotometric methods. For the detection of HPV DNA, PCR methodology was employed using most common L1 consensus primers MY 09/11 primers derived from HPV genome. HPV 16 plasmid DNA served as positive controls, whereas human placental DNA served as negative control. Amplification of β-globin gene served as internal controls to examine quality, integrity and successful amplification of cervical scrapes DNA.[12,13] [Figure 1a and b]. Cytologically abnormal HPV 16 negative cases were also tested for other HR HPV types.

Statistical analysis

Fisher's exact test was employed to find out the association between HR-HPV 16 DNA test and menopausal status as well as between HR-HPV 16 DNA and cytological abnormalities detected on Pap smears.

Results

Among 120 perimenopausal women 12 (10%) cases were positive for HPV 16 which was cytologically diagnosed as NILM (4), ASC (2), LSIL-HPV (4), HSIL (1), cancer (1). However, 108 (90%) cases were HPV 16 DNA negative consisting mainly of NILM with five cases of lower genital tract infections of *Trichomonas vaginalis* (3), candida (2), ASC (2) and LSIL-HPV (3), which were positive for LR-HPV 6/11. Cytologically koilocytotic changes are an excellent indicator of HPV infection [Figure 2a and b]. Amongst 110 postmenopausal women 14 (12.7%) cases were positive for HR-HPV 16 consisting of NILM (8), ASC (1), LSIL-HPV (2), HSIL (1), cancer (2) while 96 (87.2%) cases were negative for HPV 16 DNA consisting mainly of NILM (92) with three cases of *T. vaginalis* infestation and ASC (1), LSIL-HPV (3) were positive for HPV 6/11; however, association between HR-HPV 16 positive/negative cases and menopausal status were not statistically significant (P value = 0.5) Table 1. Cytologically abnormal HPV 16 negative cases were also tested for other HR HPV types, but none was positive; however, those cases were positive for LR HPV6/11. Cyto-histological agreement was 100% in seventeen abnormal cases (except 6 ASC) of perimenopausal and post-menopausal women. No other lower genital tract infections, except trichomonas and candida, were seen in both groups of women.

Correlation of HPV DNA test with cytologic diagnosis and menopausal status

On combining the two tests together, it was observed

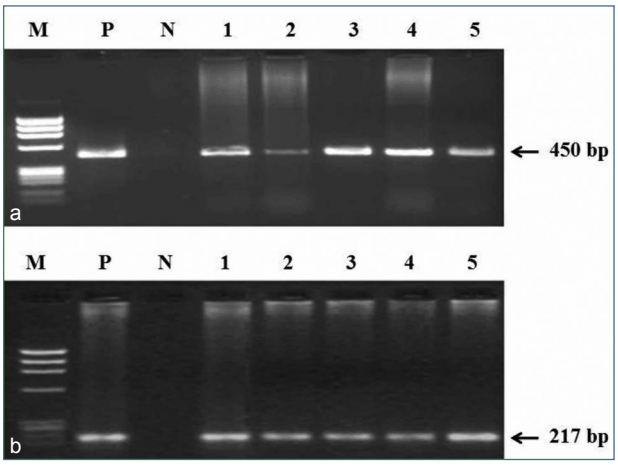

Figure 1: (a and b) Polymerase chain reaction (PCR) amplification of human papillomavirus L1 consensus primer showing amplimer of 450 bp. Lane M: Molecular weight marker, P: Positive control, N: Negative control, lane 1-5 samples showing positive by L1 PCR

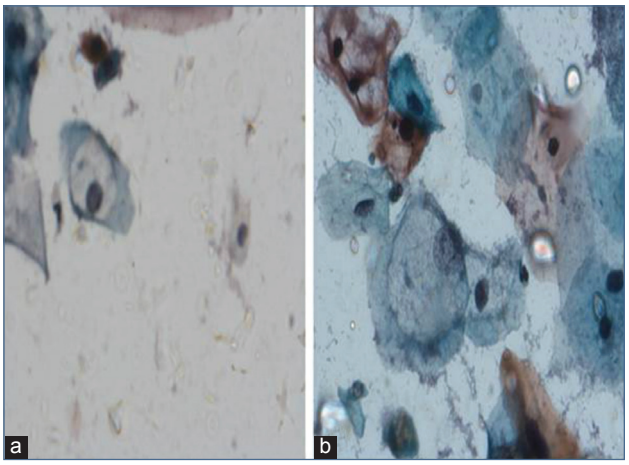

Figure 2: (a and b) The images show cells with cytoplasmic vacuolation and peripheral cytoplasmic thickening. Nuclei are slightly large and hyperchromatic (Pap, ×400)

Table 1: High-risk HPV 16 DNA test in relation to cytologic entities and menopausal status

Menopausal status of women	HPV 16 positive					HPV 16 negative			Total
	NILM (%)	ASC (%)	LSIL-HPV (%)	HSIL (%)	CA (%)	NILM (%)	ASC (%)	LSIL-HPV (%)	
Perimenopausal (45-52 years)	4 (3.3)	2 (1.6)	4 (3.2)	1(0.8)	1 (0.8)	103 (85.8) (5*)	2 (1.6)	3 (2.5)	120
Postmenopausal (53-58 years)	8 (7.2)	1 (0.9)	2 (1.8)	1 (0.9)	2 (1.8)	92 (83.6) (3*)	1 (0.9)	3 (2.7)	110
Total	12	3	6	2	3	195	3	6	230

P=0.05 non-significant. *Indicates lower genital tract infections, positive for HPV type 6/11. HPV: Human papillomavirus, DNA: Deoxyribonucleic acid, NILM: Negative for intraepithelial lesion or malignancy, ASC: Atypical squamous cells, HSIL: High-grade squamous intraepithelial lesions, LSIL: low-grade squamous intraepithelial lesions, CA: Cancer

Table 2: Association between HR-HPV 16 DNA test and cytological abnormalities in menopausal patients

Menopausal status of patients	Cases with cytologic abnormalities		Total
	HR-HPV 16 $^{+}$ve %	HR-HPV 16 $^{-}$ve %	
Perimenopausal	8 (61.5)	5 (38.4)	13
Postmenopausal	6 (60)	4 (40.1)	10
	14 (60.8)	9* (39.1)	23

*Positive for LR-HPV 6/11, *P*=1.0 non-significant. HR: High-risk, HPV: Human papillomavirus, DNA: Deoxyribonucleic acid

that in perimenopausal women association of two tests (HPV 16 and cytology) was reported in 2/4 (50%) ASC and 4/7 (57.1%) LSIL-HPV and one each HSIL and cancer while in postmenopausal women association of both test was same for ASC as 1/2 (50%) and comparatively lower in 2/5 (40%) LSIL-HPV, one HSIL and two cancers, which shows the HPV infection, decreases with the increasing age. All HSIL and cancer were positive by both test in both groups of women, which further confirms that although the Pap test is not a test for HPV, an abnormal Pap result can be the first warning sign to detect HPV infection. There were total 23 cytologically atypical/abnormal cases in both groups, of them 14 (60.8%) were positive for HR-HPV 16 and 9 (39.1%) cases were HR-HPV 16 negative, but positive for LR-HPV 6/11 [Table 2]; however, there was no statistical significance in association between HPV 16 DNA test and cytological abnormalities (*P* value = 1.00).

Discussion

All types of HPV can cause mild Pap test abnormalities, which do not have serious consequences. Identification of HR HPV type in postmenopausal women is rare. The oncogenic types HPV-16 (72%) and HPV-31 (16%) were the most commonly reported in postmenopausal women;[5] however, HPV type 16 is prevalent in India.[9]

Based on the results from the present study, the authors found that 11% of the recruited peri and postmenopausal women aging 46-58 years, had HR-HPV 16 DNA as also reported earlier,[5] which showed the prevalence of HR HPV infection as 12% in the age group of 45-54 years women. It is not clear how aging affects HPV prevalence in different age women and why there is second rise in prevalence of HPV infection in postmenopausal women, which could be a

reason for carcinoma cervix in older women.[14,15] The women who fail to eradicate their HR-HPV infection until menopause, selection of integral viral clone has taken place, driving the process towards progressing disease[16] and it is also evident that the incidence of cervical neoplasia does not decrease with the increasing age of women.

Koilocytotic changes seen in Pap smears are characteristic of the last stage of viral replication and infective genome is released as koilocytes are shed. So, it has been regarded as the most pathognomic feature and an excellent indicator of HPV infection with a high degree of specificity.[13,17] In the present study, 6/230 (2.6%) LSIL-HPV cases showed non-koilocytic changes in Pap smears, which were negative for HR-HPV 16, but positive for LR-HPV 6/11 and these changes could be due to estrogen deficiency and reparative changes.

The interpretation of smears with orangeophilic cytoplasm, nuclear variations secondary to drying and degeneration associated with atrophic vaginitis may result in cellular changes falsely interpreted as squamous atypia and/or a more severe lesion. Immature metaplastic and reparative changes are another cause of false-positive results in addition to other age-related epithelial disturbances, which include prominent perinuclear halos, nuclear hyperchromasia, variation in nuclear size and multinucleation.

HPV DNA testing by PCR permits the sensitive genetic analysis of the small amount of cells and HPV DNA test alone is significantly more sensitive than and as specific as the Pap test. The present study also revealed that in perimenopausal or transmenopausal women 4/7 (57%) cytologically detected HPV infection, were positive for HR-HPV 16 in comparison to 2/5 (40%) in postmenopausal women. Cytologically abnormal HPV 16 negative cases were also tested for other HR HPV types, but none was positive; however, those cases were positive for LR HPV 6/11.

All the cytologically abnormal cases with HR HPV variant need to be managed more aggressively compared to cytologically abnormal cases that are positive for LR variants. The 50% cytologically diagnosed ASC were positive for HR-HPV 16 in both group of women. Since HPV positivity predicted subsequent infection, testing postmenopausal patients for the virus may be a cost effective method of disease prevention in low resource settings. However by employing HPV 16 DNA testing, it is possible to exclude the HR-HPV 16 positive abnormal cases from cytologically screened population and detection and treatment of HPV related dysplastic epithelial changes can prevent the development of invasive cancer in the individual patient and require satisfactory follow-up for conservative management.

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