ORIGINAL RESEARCH PAPER
VOLUME-6 | ISSUE-6 | JUNE-2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CORRELATION BETWEEN HPV DNA TESTING AND PAP SMEAR REPORT

Gynaecology

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ABSTRACT

Objectives : We observed the correlation between HPV infection and cervival cytology in women. Methods: Women with >30years of age visiting the gynaecology outpatient with varied complaints were subjected to pap smear. 300 samples were subjected to PCR using consensus primer for HPV. The samples that were positive for HPV DNA were subsequently assessed for HPV type 16&18. *Results:* Out of 300 patients,113 (37.6%) women tested positive for HPV DNA and out of 113 HPV positive samples 23(7.66%) tested positive for high-risk HPV i.e.HPV16 (5 samples)&HPV18 (18 samples). The results showed that 24.67% women had normal smear, 9.33% had ASCUS (Atypical squamous cells of undetermined significance) , 8% had LSIL (Low grade squamous intraepithelial lesion) , 6% had HSIL (High grade squamous intraepithelial lesion) and 7% had cancer cervix. On correlation of cervical cytology with HPV DNA positivity, it was found that 100% cases of cancer cervix, 77.77% of HSIL, 54.16% of LSIL, 32.14% of ASCUS, 31.11% of inflammatory smear and 18.91% of normal report women were found positive for HPV DNA. P value of correlation is less than 0.05 which is statistically significant. Conclusion: There is significant correlation between HPV DNA positivity and cervical smear abnormality.

KEYWORDS:

Cervical cancer, HPV DNA, Pap smear

INTRODUCTION:

Human Papilloma virus (HPV) is a widely prevalent sexually transmitted virus [1,2]. Although the majority of infection are benign and transient, persistent infection is associated with the development of cervical and other anogenital cancers[3,4]. In India, about 1,00,000 women develop cervical cancer each year[5]. HPV infection is typically asymptomatic to begin with [6]. It clinically manifests as hyperplastic, hyperkeratotic warts or dysplastic lesions that may undergo neoplastic transformation [7].

Diagnosis of HPV starts with clinical suspicion and certain diagnosis can be attained by histological, cytological examinations and molecular methods looking for HPV DNA. At present, there are various molecular methods for detecting HPV DNA. Expectation from these methods is to detect high grade lesions and risk of developing high grade lesions and also to detect women with low risk.
In our study, we aimed to find the Correlation between HPV DNA positivity and Pap Smear.

MATERIALS AND METHODS:

Patients & Cervical Smear

Papanicolaou (Pap) smear cell samples were obtained randomly from new patients attending the gynaecology out patient clinic of SMGS Hospital, Government Medical College, Jammu for varied complaints. Pregnant women and women with a history of hysterectomy or conisation were excluded. Relevant data, viz; age, parity, chief complaints, clinical diagnosis, examination findings, etc. were recorded on a proforma.

Since it was a one year time bound Hospital Based Study, the calculation of sample size as in a field study was not done. It was predecided that on OPD day (once a week – Monday) every 20th patient satisfying the inclusion criteria would be included in the study. Also, every 5th Sunday used to be the OPD of the candidate. The daily OPD attendance used to be around 100. Hence, on an average 5 patient per OPD were selected and during one year (52+11) OPD days, 300 patients would be included in the study.

Cervical exfoliated cells were collected for pap smear and sent to Pathology Department, Government Medical College, Jammu for papanicolaou staining and cytology. Pap smear reporting was done according to Bethesda system of classification – 2001.

For HPV-DNA testing, cervical exfoliated cells were collected by endocervical brush in tubes and were transported under strict aseptic condition to Molecular cytogenetic laboratory of the Human Genetic Research cum Counseling Centre, Jammu University for further processing. Since PCR was the only method available in Jammu University for HPV DNA detection so it was used for the same purpose.

DNA was isolated using Phenol Chloroform method (organic method). The quantity of DNA was estimated spectrophotometrically. PCR for β actin gene was also performed for each sample, as an internal control. The DNA samples which did not show PCR product with the same were excluded.

PCR For HPV

PCR was performed on extracted DNA using primer for consensus sequence. The electrophoresis of the amplified product was done in 1.5% agarose gel. The gel was stained with ethidium bromide, to visualize the amplified PCR product. A526-594 base pair (BP) band was visualized in the positive samples for HPV on a UV trans illuminator.

Type specific PCR for HPV 16 and 18

The positive samples were subjected to type specific PCR for HPV types 16 and 18 using type specific primer for HPV 16 and HPV 18. The generated fragments were of 109 bp and 334 bp for HPV 16 and 18, respectively and were visualized on 2% agarose gels.

Statistical analysis

Statistical analysis has been performed using chi-square test . P value less than or equal to 0.05 is taken as significant.

RESULTS

Out of the 300 patients enrolled, maximum patients were in the age group of 30-40 years (57%). 113 (37.6%) women tested positive for HPV DNA by PCR, using consensus primers. 23 (7.66%) of the entire sample tested positive for high risk HPV types (16+18). HPV 16 was present in 5 (1.66%) (Fig. 1) and HPV 18 was present in 18 (6%) women (Fig. 2).

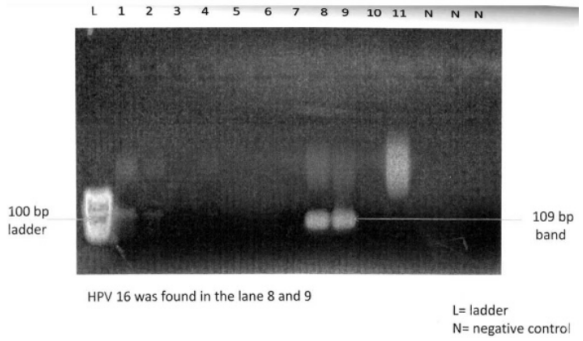

Fig. 1 Gel picture showing PCR product of HPV 16 primer in 11 patients

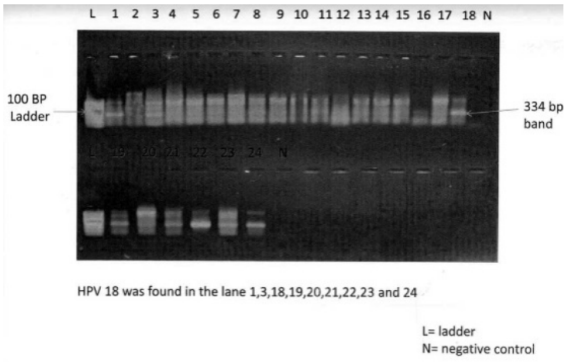

Fig. 2 Gel picture showing PCR product of HPV 18 primer in 24 patients

Table 1 shows HPV positive women in relation to cervical cytology.

Table 1 HPV positive women in relation to cervical cytology.

Pap smear report	No. of Women	No. of women positive for HPV DNA (%)
Normal	74	14 (18.9)
Inflammatory	135	42 (31.11)
ASCUS	28	9 (32.14)
LSIL	24	13 (54.16)
HSIL	18	14 (77.77)
Cancer cervix	21	21 (100)
Total	**300**	**113**

Table 2 shows Correlation of cervical cytology with HPV DNA testing.

Table 2: Correlation of cervical cytology with HPV DNA testing

HPV DNA	Pap smear abnormal	Pap smear normal	Total
Positive	57	56	113
Negative	34	123	187
Total	91	209	300

- •Chi-square test = 24.3682 P value : 0.000001
- •It is statistically significant.
- •Pap smears normal includes normal and inflammatory, and abnormal includes ASCUS, LSIL, HSIL and cancer cervix.

The sensitivity of HPV DNA testing is 62.63% , specificity is 73.20% , positive predictive value is 50.44% , negative predictive value is 81.81% , Likelihood ratio positive is 2.33 and Likelihood ratio negative is 0.51.

DISCUSSION :

Cervical cancer is a significant health problem especially in developing countries. For prevention, early detection and treatment of the preinvasive lesions that can progress to cervical cancer is very important.

Cervical cancer is the second most common cancer among women worldwide, with 83% of cases occurring in developing countries [8]. HPV infection is detected in 99.9% of the cervical cancer cases. Although the infection improves without any treatment in most women, the women with persisting HPV infection are at risk for CIN II, III and cervical cancer.

Although the Pap smear is the routinely used screening tool for cervical cancer, HPV-DNA screening in cervical samples is recommended as either an altenative or adjunvtive treatment to cervical cytology.

In the study that aimed to compare the sensitivity and specificities of HPV-DNA and Pap smear for 10154 women between the ages of 30–69, Koiopoulos et al.[9] found the sensitivity of HPV-DNA 94.6%, sensitivity of Pap smear 55.4%; specificity of HPV-DNA 94.2%, specificity of Pap-smear 96.8% for CIN II and III. When two tests are used together, sensitivity was 100% and specificity was 92.5%.

In the index study it was seen that prevalence of HPV infection increases with increasing grade of abnormality in cervical cytology. It was 18.91% in women with normal cervical cytology, 31.11% in women with inflammatory smear, 32.14% in Atypical Squamous Cells of Undetermined Significance (ASCUS), 54.16% in Low Grade Squamous Intraepithelial Lesion (LSIL), 77.77% in High Grade Squamous Intraepithelial (HSIL) and 100% in women with cancer cervix. This is consistent with Khanna A et al [10]and Varghese C [11]who also reported that HPV positivity increases with increase in abnormality in cervical cytology.

In our study prevalence of HPV 16/18 was 1.35% among controls (women with normal cytology) as compared to 20.63% among cases (Women with LSIL, HSIL and Cancer cervix). This is in accordance with Schifman MH et al [12] who concluded that the prevalence of HPV 16/18 was 2.9% among controls (Women with normal cytology) and 33.5% among cases (Women with CIN) in a case control study from USA.

In the index study, it was found that there is a significant relationship between HPV DNA testing and Pap Smear report with P value of less than 0.05. Sensitivity of the HPV DNA testing for predicting CIN and cancer cervix is 62.63% , specificity is 73.20% , positive predictive value is 50.44% , negative predictive value is 81.81% , Likelihood ratio positive is 2.33 and Likelihood ratio negative is 0.51.

In the index study, it was seen that HPV DNA testing has better specificity than sensitivity. Its negative predictive value is more than positive predictive value. So, a negative test report of HPV DNA testing is more reliable than positive report to detect any cervical lesion.

The ICMR annual report 2002-2003 mentions that various studies conducted at ICPO (Institute of Cytology and Preventive Oncology, New Delhi) have shown that HPV screening using hybrid capture technology has 75% sensitivity for detection of CIN 1 lesions and nearly 100% sensitivity for detection of high grade (CIN 2, 3) lesions with a specificity of about 83%. Though the positive predictive value was rather low (6.7%), a very high negative predictive value of 99.2% makes it an ideal tool for Indian situation where frequent screening, as is being done in western countries, is not possible. So, a potentially attractive option might be “once-in-a lifetime” screening for high risk HPV at 35 years of age.[10]

CONCLUSION:

There is a definite causal relationship between HPV and pre invasive or invasive cervical pathologies, it will continue to exist as one of the most emphasized microorganisms. Several studies have shown the usefulness of HPV-DNA testing in the triage of patients with abnormal cytology or as a primary test. If HPV-DNA tests have a reasonable cost, it will be possible to use it commonly for the socially based screening programmes.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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