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PCR screening of cervical human papilloma virus infections. Cytological and histological associations in 108 women

KEYWORDS: HPV-Human papillomavirus, PCR-Polymerase Chain Reaction NIEL-Negative for Intraepithelial lesion, IS- Inflammatory smear, CC- Chronic cervicitis, CIN-Cervical Intraepithelial Lesion CA- Carcinoma

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ABSTRACT *Human papillomavirus (HPV) infection is recognized today as the main causal factor for cervical cancer cases in the world. Integration of HPV and persistence over time, not merely infection, leads to development of high-grade precancers and cervical cancer. The current study is to evaluate cytological and histological findings with identification of cervical HPV infection using PCR technique. Chronic cervicitis was the major cervical lesion diagnosed in the present study. Maximum HPV positivity was seen in 51 to 60 year age group. In all types of cervical lesions, 31 to 33 percent showed HPV positivity which explains the importance and necessity to vaccinate all girls from 9 to 12 years. Combined with traditional Pap test, detection of cervical HPV –DNA can be very useful in cervical cancer screening programme in India.*

Introduction

In India, cervical cancer is a public health problem, with high cancer incidence and low rates of cervical cancer screening participation. Differences in cervical cancer mortality trends can be explained by the differences in screening uptake (1). Human papillomavirus (HPV) infection is recognized as the main causal factor for cervical cancer. Cervical cancer prevention now incorporates human papillomavirus (HPV)based screening with Pap testing and HPV vaccination(2). HPV infection is common, but cervical cancer is comparatively rare and usually slow to develop(3). Most HPV infections clear within a few years (4). Integration of HPV and persistence over time leads to development of cancer . Of the 14 oncogenic HPV types, HPV 16 and 18 are considered the HPV types that progress most rapidly (5). HPV testing has a higher sensitivity but lower specificity than cytology in the detection of high-grade lesions(6)

Aim

The purpose of the current study is to evaluate cytological and histological findings with identification of cervical HPV infection using PCR technique from women who underwent screening for cervical cancer in tertiary care hospital in Tiruchirappalli, Tamilnadu, India.

Material and Methods

The study was approved by the institutional ethics committee of Chennai medical college hospital and research centre and carried out at the obstetrics and gynaecology department of medical college. The study was carried out in April 2012 to March 2013.

108 women attending gynaecology outpatient department was included in the study after getting consent. We included all patients willing to undergo cervical cancer screening with detection of cervical human papilloma virus DNA. We excluded all unmarried, virgin and those not giving consent.

The structured questionnaire included information on sociodemographic characteristic's, chewing habits, reproductive, menstrual factors and use of contraception

All participants signed informed consent forms according to the recommendation of the institution ethics committee that approved the study. When indicated participating women were treated for ailments they presented with.

Gynaecologic examination and specimen collection- A total of 108 women underwent a pelvic examination. 31 women whose cervix was looking normal underwent papanicolau (PAP) smear. In addition, samples of exfoliated cells from ectocervix and endocervix were placed in 20 ml conical tubes that contained 10 ml of phosphate-buffered saline (PBS). 77 women whose cervix was looking abnormal underwent visual inspection with acetic acid and lugols iodine (VIA and VILI), followed by cervical punch biopsy

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for histopathology. In addition cervical biopsy tissue were placed in PBS containing conical tubes.

All samples were stored in iceboxes and sent daily to the laboratory of the microbiology department of Bharadidasan University, Tiruchirappalli, Tamil nadu for processing and storage. The biopsy specimens were frozen immediately at ¯20 C.The cell suspension was centrifuged at 3000g for 10 minutes at 4C, the supernatant was discarded and cell pellets were stored at ¯20 C until processing. For DNA extraction, standard SDS-K-Phenol Chloroform method was used for the biopsy samples and boiling method was used for cervical scraping. HPV DNA was first amplified using the universal MY09/11 polymerase chain reaction protocol. Type specific primers were not used due to shortage of fund.

The data were analysed using SPSS version 17.0

Results

The mean ± SD was 40.4 ± 1.96 years. 22.2% were aged 21 to 30 years, 28.7% were 31 to 40 years, 33.3 % were 41 to 50 years, and 13.88% were 51 to 60 years and 1.8% in 61 to 70 years.

Table 1 AGE WISE DISTRIBUTION OF CERVICAL LESIONS

No.	AGE in years	Number	NIEL	IS	CC	CIN	CA
1	21-30	24	4	11	9		0
2	31-40	31	4	5	20		2
3	41-50	36	1	6	27	1	1
4	51-60	15	-	-	13	-	2
5	61-70	2	-	-	2	-	-
TOTAL	108	108	9	22	71	1	5

NIEL-Negative for Intraepithelial lesion **IS**- Inflammatory smear

CC- Chronic cervicitis **CIN-**Cervical Intraepithelial Lesion **CA**- Carcinoma

Table 1 summarizes age wise distribution of cervical lesions. 8.3%, nine were negative for intraepithelial lesion (NIEL), 20.37 %, twenty two were inflammatory smear, and 65.7 % were chronic cervicitis, one case of cervical intraepithelial neoplasm and five cases of carcinoma. Chronic cervicitis was the major cervical lesion diagnosed in the present study.

Table 2AGEWISE DISTRIBUTION OF HPV STATUS

No.	AGE	Number	HPV POSITIVE	HPV NEGATIVE	HPV NOT DETERMINED
1	21-30	24	6	17	1
2	31-40	31	9	21	1
3	41-50	36	13	23	-
4	51-60	15	6	8	1
5	61-70	2	1	1	-
TOTAL	108	108	35	70	3

Table 2 summarizes age wise distribution of HPV status. 25% positive in 21 to 30 age group (6/24), 29 % in 31 to 40 years (9/31) ,36.1 % in 41 to 50 years (13/36) and 40 % in 51 to 60 years (6/15). We have seen percentage of HPV positivity increases according to age till 60 years and maximum seen in 51 to 60 year age group.

Table 3 PERCENTAGE OF HPV POSITIVITY ACCORDING TO CERVICAL LESION

LESION	TOTAL NUMBER	HPV POSITIVE	HPV NEGATIVE	HPV NOT DETERMINED	PERCENTAGE
NIEL	9	3	6	0	33.33
IS	22	7	15	0	31.80
CC	71	23	45	3	32.40
CIN	1	0	1	0	0
CA	5	2	3	0	40

Table 3 summarizes percentage of HPV positivity according to cervical lesion .33.3% in NIEL (Negative for intraepithelial lesion), 31.8 % in inflammatory smear, 32.4 % in chronic cervicitis and 40 % in carcinoma. There seems to be HPV positivity of about 31 to 33% in all age group when typing of HPV was not done. 40 % positive in carcinoma, but the number was only 5 in the present study.

Discussion

Chronic cervicitis was the major cervical lesion diagnosed in the present study. Maximum HPV positivity was seen in 51 to 60 year age group. In all types of cervical lesions, 31 to 33 percent showed HPV positivity which explains the importance and necessity to vaccinate all girls from 9 to 12 years. Overall cervical HPV prevalence was 33.3% in our study which is in agreement with the prevalence reported worldwide and is comparable with other study published by Krishna Kumar vinodhini et al(7). 32.25 (10 in 31) pap smear were scored as positive for cervical HPV which is comparable with M Zorzi study in 2013 (39.6%)(8). Combined with traditional Pap test, detection of cervical HPV –DNA can be very useful in cervical cancer screening programme in India. Population screening for cervical cancer with cytological smears has been convincingly shown to reduce cervical cancer incidence and mortality (9)The correlation of HPV with cervical lesions was seen in 29.9% cases with PAP technique and 73.4% using in-situ molecular hybridization (MH) with H3 labelled DNA probes, indicating the efficacy of the latter (10).The most critical aspect here is the increased cost of the test and

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the increased workload at colposcopy. More than 30,000 cases (about 35%) of expected carcinoma cervix cases may have been prevented by screening programmes in the UK between 1983 and 2007.The discovery of the causal role of HPV is reshaping primary and secondary prevention of Carcinoma cervix. Cheaper HPV tests are becoming available and HPV-based primary screening may at last facilitate Cervix screening in low-resource countries. In the long-term, HPV vaccination, represents the best hope for preventing Carcinoma cervix(11). Perceiving psychosocial barriers is related to lower participation in cervical cancer screening. The majority of barriers dealt with personal characteristics of women (67.9%). About 35.9% of perceived barriers referred to two categories, one referring to negative emotions evoked during Screening examination and the other focusing on negative emotions related to receiving Screening results (12). Multipronged approaches are needed to address barriers to screening, as well as identify and support conditions that encourage women's use of reproductive health services, thereby reducing incidence and mortality rates from cervical cancer(13). Cervical cancer control programs will need to be re-evaluated because the addition of HPV vaccination will make the existing approach of high-frequency screening by cytology too costly and inefficient for most public health budgets(14). The application of testing HPV genotyping and physical status based on detection of HC-II HPV DNA would be in favour of predicting the prognosis of cervical precancerosis and enhancing the screening accuracy of cervical cancer(15). Australia introduced a human papillomavirus (HPV) vaccination programme with the quadrivalent HPV vaccine for all women aged 12–26 years between 2007 and 2009 and reported a decrease in incidence of high-grade cervical abnormalities (HGA) within 3 years after the implementation of a population-wide HPV vaccination programme(16). Continued public health efforts should focus on increasing vaccine coverage in the target age groups and cervical cancer screening for women at appropriate intervals(2). Till a proper screening program is decided, triaging women with borderline cytological abnormalities and mild dyskaryosis with HPV testing would allow approximately a third of these women to be returned immediately to routine recall, and for a substantial proportion to be referred for colposcopy without repeat cytology

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