

## Original Article

# Prevalence of high risk HPV types in women referred to a colposcopy clinic in a public hospital, Chennai, South India

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To estimate the prevalence of high risk types of HPV infection in women attending colposcopy clinic in a public hospital in Chennai, South India. This was a cross sectional study. Consenting women who were attending the Gynecology Out Patient Department (OPD) and who were referred to the Colposcopy with high risk complaints were enrolled into the study. Cervical samples were taken for testing HPV types 16, 18, 31, 33, 35, 45, 52 and 58. There were a total of 55 women, aged between 24 to 60 years. High risk group women (n=12) were those women who were HIV Positive, women on steroid therapy and those who had Cervical Intraepithelial Neoplasia (CIN). 15 women (27%) were positive for HPV. Three women belonged to high risk group and five women had cancer cervix and the rest had chronic cervicitis. A total of 40 women were negative for HPV tests. Six women were HIV positive and two women were already treated for CIN2. Three women had CIN2 and One HIV positive woman had CIN3. HPV tests were negative in women with high grade lesions. All women with cancer tested positive for high risk HPV types. More studies should be done to evaluate the risk of various types of HPV in causing cancer.

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**Key words :** High risk HPV types, colposcopy clinic, CIN, Cancer cervix

Cervical cancer continues as a scourge to women worldwide, and still remains a major cause of mortality among women. The Human Papilloma Virus (HPV) has been proven as the causative agent, and causes cancer along with co factors. HPV is the most common sexually transmitted infection (STI). Over 100 types of HPV have been identified which affect humans and of these at least 40 types infect the genital mucosa. Taking into consideration their association with cancer, they have been grouped into high risk, intermediate risk and low risk groups.

Of the many different types of HPV, some can cause health problems including genital warts and cancers. HPV infection is the most important risk factor for cervical intraepithelial neoplasia and invasive cervical cancer<sup>1</sup>.

There were an estimated 527,600 new cervical cancer cases and 265,700 deaths worldwide in 2012. It is the second most commonly diagnosed cancer and third leading cause of cancer death among females in less developed countries<sup>2</sup>. Cervical cancer is the second most common

female cancer in women aged 15 to 44 years in the world<sup>3</sup>. Every year in India, 122,844 women are diagnosed with cervical cancer and 67,477 die from the disease<sup>4</sup>. In India the peak age for cervical cancer incidence is 55-59 years<sup>5</sup>.

The persistence of HPV infection has been identified in almost all cervical cancers. The precancerous condition of cervix is called as cervical intraepithelial neoplasia (CIN). The natural history of HPV induced carcinogenesis has been proposed as initial infection with HPV of the cervical epithelium, usually unnoticed as it is asymptomatic in most women. More than 75% of all women are infected with HPV in their life time. The HPV infection causes changes in the cervical epithelium in a small group of less than 30% of infected women and can be detected by cytology. Though the infection is cleared spontaneously in most women, within one to three years, it has been observed that HPV infection persists in a small percentage of 10 to 20% of women and this persistence caused the abnormal changes in the cervical epithelium, which may become progressive and is termed as CIN. The initial low grade lesions affecting a third or less of the cervical epithelium slowly progress on to high grade lesions affecting more than half the thickness of the cervical epithelium and later on, by breaking the basement become invasive cancer.

HPV infection is typically asymptomatic to begin with<sup>6</sup>. The transmission occurs prior to any clinically detected expression of the virus. HPV infects the basal cells of the

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epithelium<sup>7</sup>. The virions assemble in the nucleus and are subsequently shed from keratinocytes. There is proliferation of all the epithelial layers except the basal epithelial layer. The virus has an incubation period of 3-4 months<sup>8</sup>. The strength of the HPV cervical cancer relationship is even greater than the association between smoking and lung cancer and other well established causal relationships in cancer<sup>9</sup>. Based on the epidemiologic classification the high-risk HPV types are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82. Low-risk types are 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP 6108<sup>10</sup>. HPV types 16, 18, 45 and 31 are considered to be the most important oncogenic types. Subtypes 16 and 18 are the causative agents of more than 50% of cervical pre-cancerous lesions, and more than 70% of cervical cancer cases<sup>11</sup>.

Papanicolaou staining (Pap smear) is a common screening method for cervical cancer in India. Simple screening tests of visual inspection of cervix after application of acetic acid (VIA) and Lugol's iodine (VILI) are now most commonly used and cost effective. A clinical trial in rural India found that a single round of HPV testing reduced the number of cervical cancer deaths by about 50%<sup>12</sup>. PCR is now the gold standard test for HPV research. HPV DNA testing was both more sensitive and specific than Pap cytology and VIA<sup>13</sup>.

The natural history of HPV infection has been more clearly defined with PCR. This methodology has overcome the problem of misclassification of HPV status that initially confused the scientific community regarding the role of HPV in cervical cancer<sup>14</sup>.

The aim of this study was to find out the prevalence of high risk HPV types in women referred to colposcopy clinic in a tertiary care, public teaching hospital in South India.

#### MATERIALS AND METHOD

This was a cross sectional study. Women were referred to colposcopy clinic from Gynecology outpatient department (OPD) and from the wards. Referral to colposcopy clinic was for various complaints like abnormal vaginal discharge, women with high risk background such as HIV positive women, women on steroid therapy, women with family history of cancer cervix, symptomatic women hailing from areas with high incidence of cancer cervix, history of abnormal bleeding, abnormal appearance of cervix, abnormal pap smear reports and for colposcopic evaluation prior to gynecological surgical procedures<sup>15-17</sup>.

Women were selected randomly and enrolled into the study after obtaining informed consent<sup>16,18</sup>. Routinely all women referred to the colposcopy clinic were examined and full colposcopic assessment of the anogenital area was done using 5% acetic acid (VIA). The observed colposcopic appearance was recorded for each participant. Punch biopsy was taken for histological examination for

fifty two women with abnormalities detected under colposcope. Three women did not require cervical biopsy.

Cervical swab was obtained from women who were enrolled into the study and tested for HPV types 16, 18, 31, 33, 35, 45, 52 and 58. The samples were transported into the Department of Experimental Medicine, The Tamilnadu Dr MGR Medical University for further processing. DNA was extracted from the sample using AmpliGenei HPV Detection Kit. Polymerase Chain Reaction was performed with 21.6 µl HPV amplification mix with 0.4 µl of Genei Hotstart Taq polymerase and 3 µl of DNA was added into the tube. The reaction conditions were initial denaturation at 95°C for 5 minutes for the first cycle and 94 °C for 1 minute, 62°C for 1 minute and 72°C for 1 minute for 10 cycles and was followed by 94°C for 45 seconds, 58°C for 45 seconds and 72°C for 45 seconds for 35 cycles and the extension was 72°C for 5 minutes. The positive and negative controls were used along with each assay. The amplified products were run on 2% agarose gel electrophoresis and the positive bands were visualized on UV spectrophotometer. An amplification product of size between 230-270 base pair indicated infection with one or more of eight oncogenic HPV types. The HPV types tested were Type 16, 18, 31, 33, 35, 45, 52 and 58.

#### DISCUSSIONS AND RESULTS

There were a total of 55 women who aged between 24 to 60 years. Nearly 47.7% of the women had no formal education and did not know to read or write. However 37% had primary education, 13.8% had secondary education and one woman held a degree qualification. Fifteen women had history of early marriage and child birth and history of coital activity of nine years, and more. Six women were referred from high incidence areas of cancer cervix. Fifteen women were referred for abnormal appearance of cervix, and nine women for abnormal vaginal discharge.

A high risk group was identified comprising of HIV positive women, women living with HIV positive spouse, women on steroid therapy and women with previous history of CIN (n=12). More than one risk factor was elicited in 60% of the women. One woman was on steroid therapy. Eight women were HIV positive and five of them were on antiretroviral therapy. One woman whose husband was HIV positive was tested negative for HIV and HPV but diagnosed with CIN1. Six HIV positive women tested negative for HPV, though two of them had high grade lesions in cervix and biopsy cervix showed CIN2 in one and CIN3 in the other. CIN 1 was reported in one HIV positive woman. Two women, who had been treated for CIN in the past, also tested negative for HPV and also cervical lesions.

A total of 15 (27%) women tested positive for HPV (Table 1) (Fig 1). A population study from Eastern India

Table 1 — HPV Positive Group				
Age in years	PAP smear	HPV test	Colposcopy diagnosis	HPE reports
35	LSIL	Pos	Cancer cervix	Squamous cell cancer
45	NSIL	Pos	Chronic cervicitis	Chronic cervicitis
42	NSIL	Pos	Chronic cervicitis	Chronic cervicitis
24	NSIL	Pos	Chronic cervicitis	Chronic cervicitis
32	NSIL	Pos	High grade lesion	CIN2
40	NSIL	Pos	Chronic cervicitis	Chronic cervicitis
35	CACX	Pos	Cancer cervix	Squamous cell cancer
38	NSIL	Pos	Chronic cervicitis	Chronic cervicitis
44	NSIL	Pos	Cancer cervix	Squamous cell cancer
40	NSIL	Pos	High grade lesion	CIN3
58	BLEED	Pos	Cancer cervix	Squamous cell cancer
28	NSIL	Pos	Low grade lesion	Chronic cervicitis
HIGH RISK GROUP				
45	NSIL	Pos	Chronic cervicitis	Chronic cervicitis
39	---	Pos	Cancer cervix	Squamous cell cancer
35	LSIL	Pos	High grade lesion	CIN2

HPV tests were positive in 15 women. Five women had Cancer cervix, One woman had CIN1. Two women had CIN2 and seven women had chronic cervicitis. The last three women belonged to high risk group. Row one The woman was On steroid therapy for three years. Second and third row Both women were HIV positive and on ART. NSIL - Negative for squamous intra epithelial lesion. LSIL - Low Grade Squamous Intraepithelial lesion. HSIL - High Grade Squamous Intra Epithelial Lesion.

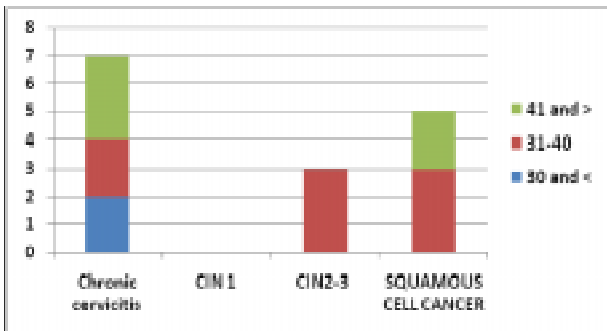


Fig 1— HPV positive group - diagnosis by cervical biopsy according to age group  
 HPV tests were positive in 15 women. The largest group of women who tested Positive for HPV were aged between 31 to 40 years. High grade CIN, was diagnosed in 37.5% and carcinoma of cervix was diagnosed in 37.5% of women in this age group.

has showed the prevalence of HPV among women without cervical cancer to be 9.9%<sup>19</sup>. While HPV prevalence among cervical cancer patients in India is high as 91.7%<sup>20</sup>.

In our study three (3/15) HPV positive women were belonged to high risk group and five women (5/15) had cancer cervix and the remaining seven women had chronic cervicitis.

Though seven women with an average age of 36.3 years, had high grade lesions of cervix, (CIN 2, n=4, CIN 3, n=3) only three women were positive for HPV. The average age of women with cancer was 42.2 years (n=5) and all were diagnosed with squamous cell carcinoma of cervix and tested positive for HPV. HPV positive tests were

recorded in seven women with chronic cervicitis (average age was 37.2 years).

A total of 40 women were negative for HPV tests (Table 2) (Fig 2). Six women were HIV positive and two women were already treated for CIN2. Three women had CIN2 and One HIV positive woman had CIN3 (Table 2)(Fig 2).

**CONCLUSION**

All women with cancer tested positive for high risk HPV types. HPV tests were negative in women with high grade lesions, even in HIV positive women. More studies should be done to evaluate the risk of various types of HPV in causing cancer cervix, and precancerous lesions of cervix.

Table 2 — HPV Negative Group		
No of women	Average age of women	HPE reports
27	41	chronic cervicitis
6	36.6	CIN1
2	40	CIN 2
2	34	CIN3

Forty women tested negative for HPV Tests. Three women had normal study of cervix as diagnosed under Colposcope and did not require biopsy. For twenty seven women, histopathological examination of biopsy tissue of cervix reported, chronic cervicitis and confirmed that there was no evidence of CIN. HPE reports confirmed CIN in Ten women, with negative HPV tests. Six women had CIN 1, Two women had CIN2 and two women had CIN3.

Table 2A — High Risk Group With HPV Negative Tests					
Age	Pap Smear	Colposcopy Diagnosis	HPV Result	High risk factor	HPE Report
42	NSIL	Chronic cervicitis	Neg	HIV +	CIN1
35	NSIL	Chronic cervicitis	Neg	Old CIN 2	Chronic cervicitis
39	NSIL	Chronic cervicitis	Neg	Old CIN	Chronic cervicitis
41	NSIL	Chronic cervicitis	Neg	HIV +	Chronic cervicitis
47	NSIL	Chronic cervicitis	Neg	HIV +	Chronic cervicitis
33	LSIL	Chronic cervicitis	Neg	HIV+spouse	CIN1
29	ASCUS	LSIL	Neg	HIV +	CIN3
24	NSIL	HSIL	Neg	HIV +	Chronic cervicitis
43	NSIL	Chronic cervicitis	Neg	HIV +	CIN2

HPV tests were negative in two HIV positive women with high grade lesions.

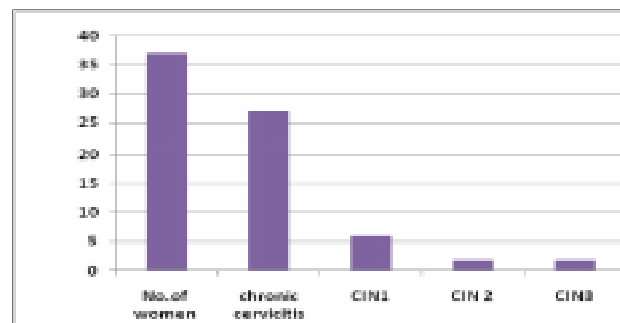


Fig 2 — HPV negative group - diagnosis by cervical biopsy  
 The average age of women grouped according to diagnosis by cervical biopsy ranged from 34 years to 41 years. The average age of all 37 women was 38 years.

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