Original Research Article

# Prevalence of oncogenic human papillomavirus (HPV 16/18) infection, cervical lesions and its associated factors among women aged 18–40 years

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## Abstract

**Background:** Human papillomavirus (HPV) infection is considered as the major risk factor for the development of cervical cancer, second most frequent cancer in India. However, the magnitude of the problem and the associated factors remain unrevealed in the Puducherry region. This study aimed to determine the prevalence of HPV infection and factors contributing to the progression of HPV infection to cervical cancer. **Methods:** It was a prospective cross-sectional study. Women of the reproductive age group aged between 18 to 40 yrs attending the obstetrics and gynaecological outpatient department at Sri Venkateswaraa Medical College Hospital and Research Centre, Ariyur, Puducherry were included in the study. This study involved 150 outpatients. The study was performed over a period of 1 year, between November 2013 to November 2014. Socio demographic and clinical data were collected using a pretested questionnaire and detection of HPV infection was done using HPV test (OncoE6$^{TM}$ Cervical Test) specific to HPV16/18 in cervical swabs. **Results:** The study group populations were aged between 18 to 40 years of age and were married. The demographic data shows that most of the patients (n=24) were 40 years of age followed by patients aged 35 years (n=17). Most of the patients (n=96) were in the age group ranging between 30 to 40 years of age. None of the patients in our study group accepted smoking habit or multiple sexual partners. Majority of the study group population had their first sexual exposure at 19 years of age. Most of the study group women were multiparous. In this study, 17 patients were on oral contraceptive pills. The study population ranged between 18 to 40 years of age with a mean of 31.92 years median of 32 years. **Conclusion:** There was a relatively low prevalence of oncogenic HPV 16/18 and VIA-positivity in women attending hospitals in the Puducherry Region. Early age sexual contact, high parity, and being uneducated/low level of education were independently associated factors with HR-HPV infection and development of cervical lesions, highlighting the importance of prioritizing the limited HPV testing to those risk groups.

**Keywords:** HPV, Cervical Lesions, HPV 16/18, Prevalence



**Inroduction**

Cervical cancer is the fourth most common site of cancer among women based on World Health Organization (WHO) cancer fact sheet update in February 2015. It is one of the most common malignancies in women worldwide and is the commonest cause of death in developing countries. Cervical cancer is the second most common cancer in women living in developing countries based on WHO Human Papilloma Virus (HPV) and cervical cancer fact sheet in March 2015.

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WHO fact sheet (updated in November 2013) on Sexually Transmitted Infections (STI) states that more than 1 million people are estimated to acquire a STI every day which could be due to more than 30 different types of bacterial, viral or parasitic pathogens. Of these, 8 pathogens are more contagious and cause high incidence of disease. Of the 8 pathogens, 4 are viral infections which include HPV, hepatitis B, herpes and Human Immunodeficiency Virus (HIV).

Human Papilloma Virus, the most common viral infection of the reproductive tract, is the main cause for the development of cervical cancer. HPV infection was estimated to cause 5,30,000 cases of cervical cancer and 2,75,000 cervical cancer deaths each year. Nearly all (99%) cervical cancer cases are associated with genital HPV infection. Persistent infection with HPV is recognized as an inevitable cause for the development of cervical cancer, however majority of the women with HPV infection do not develop cervical cancer. HPV infection is also linked with anal, vulval, vaginal, penile and oropharyngeal tumors, and also causes genital warts.

Genital HPV infections are usually transmitted through genital contact and most people acquire HPV infection shortly after becoming sexually active. Most of the HPV infections are asymptomatic, transient with spontaneous resolution and more than 90% clear within 2 years. Clearance usually occurs in the first 6 months after infection. However a small proportion of the high-risk HPV types can cause persistent infection and risk the development of cervical intraepithelial neoplasia and invasive cervical cancer. It is estimated to take 15 to 20 years for development of cervical cancer in immunocompetent and only about 5 to 10 years in immunocompromised women [1].

There are more than 100 types of HPV, which are grouped as low-risk (nononcogenic) and high-risk (oncogenic) types. Predisposing factors to cervical cancer development includes persistent infection with oncogenic HPVs, immunodeficiency, high viral load and cofactors like smoking, multiple sex partners and poor nutrition [2, 3].

Based on the data published on HPV and related cancers in India by ICO (InstitutCatalad' Oncologia) information centre on HPV and cancer (Fact Sheet 2014), an estimate of 122844 new cervical cancer cases and 76477 deaths are noted annually. The crude cervical cancer incidence rate is about 20.2%. Cervical cancer is the second most frequent cancer among Indian women and among women aged between 15 and 44 years of age. At a given time, 5% of the women have cervical HPV 16/18 infection and these two genotypes together account for 82.7% of invasive cervical cancers. The HPV prevalence rate in women with normal cytology (n=35349) is about 7% (95% Confidence Interval). Factors contributing to cervical cancer (cofactors) included smoking (prevalence-2.8%), total fertility rate (2.8 live births per women) and oral contraceptive use (3.1%).

**Aim & Objectives**

Hence the present studt, to find out the prevalence of human papilloma virus infection with oncogenic genotypes 16 and 18 in women of reproductive age group (18-40years).

**Materials and methods**

**Study design**

A prospective cross-sectional study

**Study group**

Women of the reproductive age group aged between 18 to 40 yrs attending the obstetrics and gynaecological out patient department at Sri Venkateswaraa Medical College Hospital and Research Centre, Ariyur, Puducherry were included in the study.

**Study group volume/population**

This study involved 150 outpatients

**Study duration**

The study was performed over a period of 1 year, between November 2013 to November 2014.

**Patient Selection**

**Inclusion criteria:**

1) Women aged between 18 to 40 years attending the Obstetrics and gynaecological out patient department
2) Women who provided informed consent

**Exclusion criteria**

1) Women younger than 18 years and older than 40 years of age
2) Women who presented during their menstrual period
3) Women with history of cervical carcinoma
4) Women on treatment or during follow up period for cervical carcinoma

Institutional Ethical Committee and scientific research committee clearance was obtained before the commencement of the study.

An informed consent was obtained and detailed questionnaire were given to the subjects included in the study.

**Sample collection, storage & processing**

HPV sample collection kit for HPV DNA PCR containing spatula, cyto-brush & tube containing 100% ethanol were used. Equipment's:

1) Metal Speculum of suitable size
2) Wooden Spatulas for ectocervical sample
4) Cytobrush for endocervical sample
5) Gloves
6) Microscope slides
7) Fixatives for pap smear
8) Absolute Ethanol
9) HPV DNA extraction kit

**Sample collection - Overview**

The sample was collected in Obstetrics and Gynaecology out patient department in Sri Venkateswaraa Medical College Hospital and Research Centre. Sample collection was done on the basis of inclusion and exclusion criteria. The importance and purpose of this study was explained to the patient and informed consent was obtained from the patient before the procedure. The clinical details regarding this topic were collected. Pap smears was done free of cost for the patient and processed in pathology department.

**Procedure for Pap smear**

Before starting of the procedure, labeling of slide and the tube was done. Under aseptic precautions sampling was performed.

1) Following insertion of the vaginal speculum, the spatula was rotated 360 degrees after ensuring its proper placement in the cervix and the pap smear was taken and processed.
2) Then cyto-brush was rotated in the endometrial canal and all the cervical tissue scraped using spatula is picked up by the cyto-brush.
3) The cyto-brush after sample collection is cut and dropped into the 1.5 ml appendorfs tube containing absolute ethanol.
4) The tube containing cyto-brush is then stored at -20 degree Celsius in deep freezer till testing.

**Results**

**Demographic data**

In this study, a total study population of 150 subjects was enrolled. The study group populations were aged between 18 to 40 years of age and were married. Table-1 shows the age distribution and the maximum number of subjects involved in this study were 40 years of age.

The demographic data shows that most of the patients (n=24) were 40 years of age followed by patients aged 35 years (n=17). Most of the patients (n=96) were in the age group ranging between 30 to 40 years of age.

The study population ranged between 18 to 40 years of age with a mean of 31.92 years median of 32 years. Table-2 shows the range, mean, median and mode of the study group age.

Of the total, 97% of women were housewives and the remaining 3% were not housewives and were involved in some sort of labor. Figure-7 shows the distribution of the study group based on their occupation.

In this study, 17 patients were on oral contraceptive pills. Figure-2 shows the percentage distribution of women who were on oral contraceptive pills.

None of the patients in our study group accepted smoking habit or multiple sexual partners. Majority of the study group population had their first sexual exposure at 19 years of age. Most of the study group women were multiparous.

**Table-1: Age Distribution**

| S.no | Age in yrs | Number of samples |
|---|---|---|
| 1 | 40 | 24 |
| 2 | 39 | 6 |
| 3 | 38 | 13 |
| 4 | 37 | 9 |
| 5 | 36 | 0 |
| 6 | 35 | 17 |
| 7 | 34 | 1 |
| 8 | 33 | 4 |
| 9 | 32 | 5 |
| 10 | 31 | 11 |
| 11 | 30 | 6 |
| 12 | 29 | 5 |
| 13 | 28 | 5 |
| 14 | 27 | 6 |
| 15 | 26 | 9 |
| 16 | 25 | 6 |
| 17 | 24 | 5 |
| 18 | 23 | 2 |
| 19 | 22 | 2 |
| 20 | 21 | 3 |
| 21 | 20 | 8 |
| 22 | 19 | 1 |
| 23 | 18 | 2 |

**Table-2: Age Distribution**

| | Mean | Median | Mode | Range |
|---|---|---|---|---|
| Age (in yrs) | 31.92 | 32 | 40 | 18-40 |

**Table-3: Sensitivity and Specificity for Pap smear**

| Variables | Value | 95% Confidence Interval |
|---|---|---|
| Sensitivity | 0.01333 | 0.001619 to 0.04730 |
| Specificity | 1 | 0.9757 to 1.000 |
| Positive predictive value | 1 | 0.1581 to 1.000 |
| Negative predictive value | 0.5034 | 0.4453 to 0.5613 |

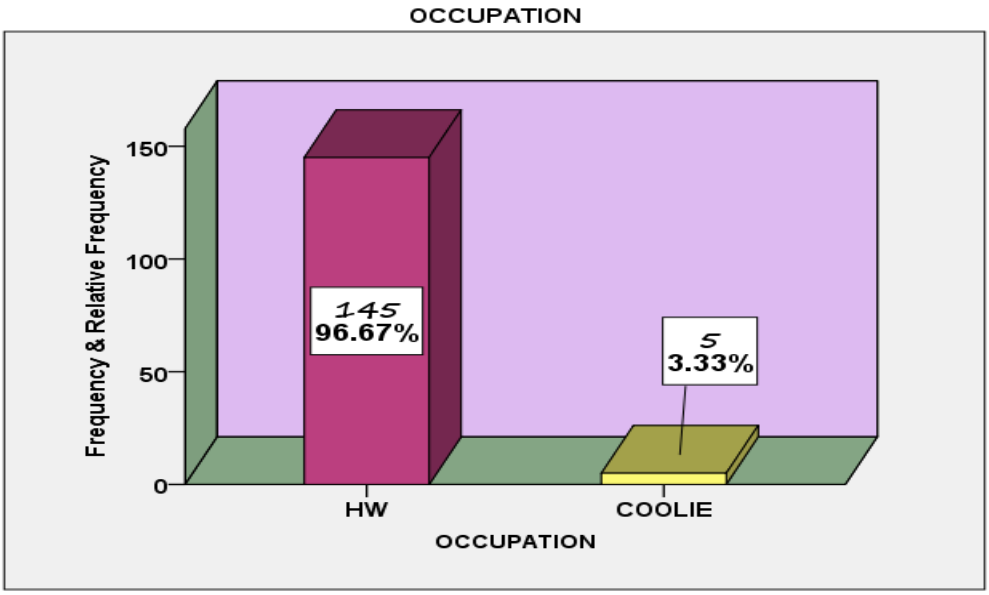


**Fig 1: Occupation distribution**

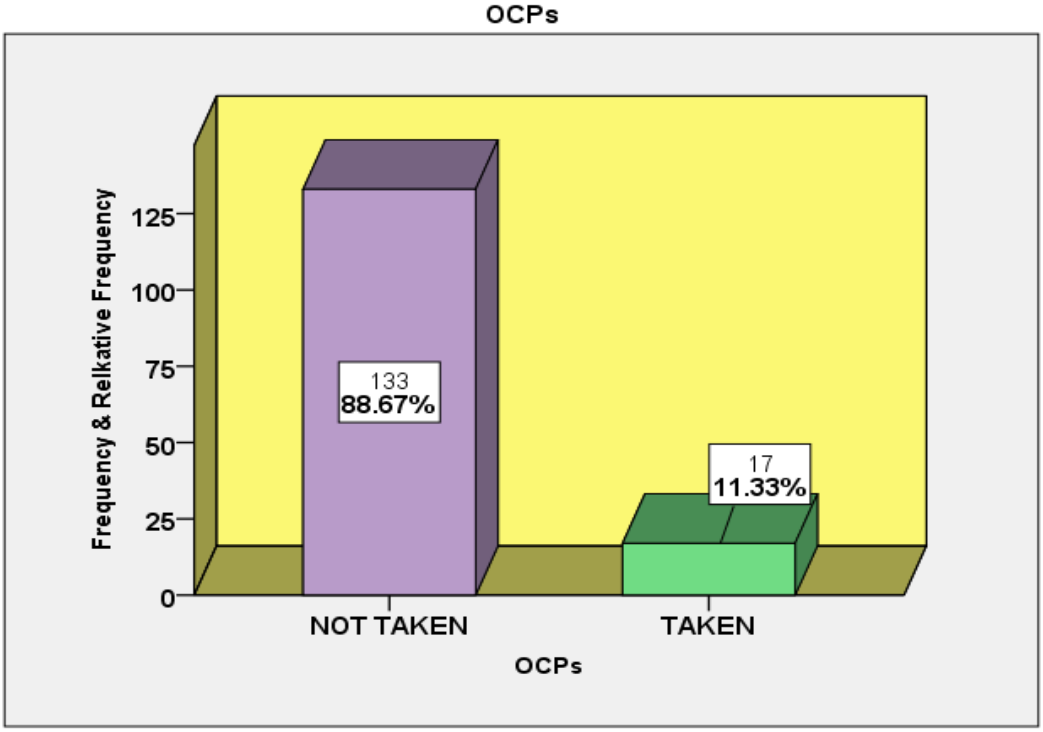


**Fig 2:Distribution of Oral contraceptive pills intake**

**Discussion**

Cervical cancer is the leading cause of cancer in Indian women and it is the fourth most common cancer in women worldwide[4]. Persistent cervical infection with HPV is recognized as an essential causative agent for cervical cancer development. Cervical cancer occurs mainly during the early reproductive age group of the women. The incidence is high between 30 to 34 years of age and peaks at 55 to 65 years. The median age is about 38 years. More than 80% of the sexually active women are estimated to acquire genital HPV before 50 years of age[5].

Grace Nirmala Jet al[6] in their study showed HPV genotype 16 and 18 to be the most common types prevalent (HPV 16 - 57% and HPV 18 - 18%) in cervical cancer patients in patients from Trichy and Coimbatore districts of Tamilnadu state in India[6]. Of these two, HPV 16 was the most prevalent genotype. In 2009, it was reported by Berlin Grace VM that in India the highest incidence of cervical cancer is in Chennai. They observed high prevalence of HPV 16 in cervical cancer but they found HPV 18 type to be more oncopotent (66.6% dysplasias)[7]. Dutta S et.al estimated the human papilloma virus prevalence in women without cervical cancer in eastern India and found out it to be 9.9% for any type. In contrary to the findings described in other studies, the prevalence of HPV 18 was greater than that of HPV 16 in their study. Moreover type 16 was associated with high oncogenicity[8]. Numerous studies in literature have stated HPV genotype 16 and 18 to be the most prevalent type causing cervical infection. The national statistics on HPV prevalence is shown in the WHO factsheets.

These variability in the prevalence rate of type specific HPV (type-16&18) prompted us to look into the prevalence of HPV type 16 and 18 in women visiting the outpatient of obstetrics and gynaecology department in our institution. Also we were interested in knowing the regional prevalence of HPV type 16 and 18 in our area and in women of reproductive age group. Over a period of about one year we evaluated about 150 patients. We decided to screen the patients at earlier age to reduce the risk of cervical cancer by detecting lesions which has the potential to become invasive cervical cancer. We restricted the study age group to between 18 to 40 years of age. The study samples were collected from each patient and were processed as described in the materials and methods. HPV testing has higher sensitivity when compared with cytology in detecting existing high-grade cervical lesions.

The median age was about 32 years in our study. Women who presented during their menstrual period, women with history of cervical carcinoma and women on treatment or during follow up period for cervical carcinoma were not included in this study. Sauvaget et al studied prevalence and determinants of HPV in middle aged Indian women. They studied women aged between 30 to 59 years of age and the prevalence was estimated to be 10.3%. They also found that HPV infection to be associated with low socio-economic status, increasing age, low literacy, manual work, early age at first sexual intercourse and widows or divorce[9]. But in our study population most of them were housewives. In common they were of low education level similar to the above mentioned study.

In their study 'Demographic characteristic of HPV infection in women - a hospital based study from Guwahati, India' the authors described the association of HPV prevalence women with the parity. They found out that women with 5children or more showed higher HPV prevalence (21.7%) compared to those who had 1-2 children in whom the prevalence was 4.7%[10].

Aggarwal Ret al studied the high-risk (HR) HPV prevalence in women with benign cervical cytology. They also evaluated the possible association of HR-HPV infection with various demographic data. The prevalence rate for HPV DNA was 36.8% and for HR-HPV DNA was 8.2%. They found high prevalence in rural patients and in poorly educated patients. They were not able to find any of HPV prevalence with age, parity and age at marriage[11]. Similarly in a study by Bhatla N and others done in All India Institute of Medical Sciences (AIIMS) from October 2005 to October 2007, they found no significant association of HPV infection or abnormal histopathology with older age (>35 years), high parity, oral contraceptive use and smoking[12,13].

In a review article, Sreedevi Aet al studied the epidemiology of cervical cancer in India recently (2015). They found the peak incidence of cervical cancer to be between 55 to 59 years of age. Similar to most other studies HPV types 16 and 18 were identified in patients with cervical cancer. They identified early age at marriage, poor hygiene, low socio-economic status, multiple sexual partners, multiple pregnancies, use of oral contraceptives and lack of awareness as the epidemiological risk factors[14]. In our study about 11.33% (n=17) of the study population were on oral contraceptive pills while the rest (n=133) were not taking OCP's. An association of OCP's with HPV prevalence was not established in our study.

Now and then, studies have reported probable association of OCP's intake and the length of its intake with development of cervical cancer in women with HPV infection. In a multi-centric study, Moreno V and colleagues studied the effects of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection. They concluded that long-term oral contraceptive use could be a cofactor that increases the risk of cervical cancer by up to four-fold in women who are positive for cervical HPV DNA. Since worldwide statistics was not widely available they suggested in motivating women on long-term oral contraceptives to undergo cervical screening programmes[15].

Even WHO gave safety information regarding the reports on association between increasing risk of cervical cancer and duration of use of oral contraceptives in women with HPV. It was reported that there is a threefold increase in risk following 5 to 9 years of oral contraceptive use and about a fourfold increase after 10 or more years of OCP's intake. However most of these studies original studies were carried out in developing countries where no adequate cervical screening programme is in practice. Hence they suggested that all sexually active women, especially those on long-term oral contraceptives, to have regular cervical smears. Also in majority of women who are on OCP's the benefits outweigh the risks[16].

Srivastava et al[17] showed demographic factors like high parity, rural residence, low socioeconomic status, elderly age and postmenopausal age group women were associated with development of cervical cancer. They suggested screening programs and HPV vaccination targetting women of low socioeconomic status and those living in rural areas. In our study majority of the study population were residing in rural areas.

The prevalence rate based on PCR was nil and no comment on the association of demographics with the HPV prevalence be made.

**Conclusion**

However further studies with large sample size is needed to determine the prevalence of high-risk HPV genotypes 16 and 18 in our region and to ascertain the sensitivity of HPV DNA.

Our study shows that prevalence of oncogenic HPV 16/18 and cervical lesions among women visiting hospitals for gynecology and family planning services are low. This is possibly because of (i) exclusion of pregnant women with confirmed cervical cancer and pregnancy from the study, and (ii) poor sensitivity of VIA. The present study indicates testing for HPV together with VIA improves early detection of high risk women for successful cervical cancer screening programs. However, more research is needed to better understand VIA positivity among HPV infected women. This study also identifies early age sexual contact related to early marriage, high parity and low level of education as independent associated factors to develop cervical lesions, supporting the importance of prioritizing the limited HPV screening test to those risk groups as well as vaccinating female children at age of 7–9 years.

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**References**

1. WHO website [Internet].March 2015:Fact sheet N°380. http://www.who.int/mediacentre/factsheets/fs380/en/
2. Gnanamony M, Peedicayil A, Abraham P. An overview of human papilloma viruses and current vaccine strategies.Indian J Med Microbiol. 2007; 25 (1):10-7.
3. Munoz N. Human papillomavirus and cancer: the epidemiological evidence. J Clin Virol. 2000 Oct; 19(1-2):1-5.
4. Kaarthigeyan K. Cervical cancer in India and HPV vaccination.Indian J Med PaediatrOncol. 2012 Jan; 33(1):7-12.
5. Singh N. HPV and Cervical cancer - prospects for prevention through vaccination. Indian J Med PaediatrOncol. 2005;26:20-3.
6. Grace Nirmala J, Narendhirakannan RT. Detection and genotyping of high-risk HPV and evaluation of anti-oxidant status in cervical carcinoma patients in Tamil Nadu State, India - a case control study. Asian Pac J Cancer Prev. 2011; 12(10) :2689-95.
7. Berlin Grace VM. HPV type 18 is more oncopotent than HPV16 in uterine cervical carcinogenesis although HPV16 is the prevalent type in Chennai, India. Indian J Cancer. 2009 Jul-Sep;46(3):203-7.
8. Dutta S, Begum R, MazumderIndra D, Mandal SS, Mondal R, Biswas J, et al. Prevalence of human papillomavirus in women without cervical cancer: a population-based study in Eastern India. Int J GynecolPathol. 2012 Mar;31(2):178-183.
9. Sauvaget C, Nene BM, Jayant K, Kelkar R, Malvi SG, Shastri SS, et al. Prevalence and determinants of high-risk human papillomavirus infection in middle-aged Indian women. Sex Transm Dis. 2011 Oct;38(10):902-6.
10. Sarma U, Mahanta J, Borkakoty BJ, Talukdar KL, Gogoi R, Yadav K. Demographic characteristic of HPV infection in women - a hospital based study from Guwahati, India. National journal of medical research.2013;3(1):9
11. Aggarwal R, Gupta S, Nijhawan R, Suri V, Kaur A, Bhasin V, et al. Prevalence of high - risk human papillomavirus infections in women with benign cervical cytology: A hospital based study from North India. Indian J Cancer. 2006Jul-Sep;43(3):110-6.
12. Bhatla N, Puri K, Joseph E, Kriplani A, Iyer VK, Sreenivas V. Association of Chlamydia trachomatis infection with human papillomavirus (HPV) & cervical intraepithelial neoplasia - A pilot study. Indian J Med Res. 2013 Mar; 137(3):533-9.
13. Franceschi S, Rajkumar T, Vaccarella S, Gajalakshmi V, Sharmila A, Snijders PJ, et al. Human papillomavirus and risk factors for cervical cancer in Chennai, India: a case-control study. Int J Cancer. 2003 Oct 20; 107(1):127-33.
14. Sreedevi A, Javed R, Dinesh A. Epidemiology of cervical cancer with special focus on India. Int J Womens Health. 2015 Apr 16; 7:405-14.
15. Moreno V, Bosch FX, Muñoz N, Meijer CJ, Shah KV, WalboomersJM, et al. International Agency for Research on Cancer. Multicentric Cervical Cancer Study Group. Effect of oral contraceptives on risk of cervical cancer in women with human
16. WHO Drug Information Vol. 16, No. 2, 2002 [Internet]. http://apps.who.int/medicinedocs/en/d/Js4950e/
17. Srivastava S, Shahi UP, Dibya A, Gupta S, Roy JK. Distribution of HPV Genotypes and Involvement of risk factors in cervical lesions and invasive cervical cancer: A study in an Indian population. Int J Mol Cell Med. 2014 Spring; 3(2):61-73.