

# Non-invasive saliva-based screening of high-risk Human Papilloma Virus 16 and 18 in healthy young adults and creating awareness about its vaccination

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## ABSTRACT

**Context:** Human Papilloma Virus (HPV) has not only been linked with cervical cancer but also a key player in other types including oral cancer. Vaccine against HPV has shown promising outcomes in protection against cervical cancer. It is suggested that the same vaccine may be a safeguard against oral cancer as well. Since prevalence of oral cancer is on rise because of various reasons besides high-risk sexual behavior, its prevention becomes equally important. **Aim:** Study aimed at screening saliva samples of healthy young adults to detect the presence of HPV with an intention to increase awareness regarding HPV and its vaccination. **Settings and Design:** The study was executed in the department of Biochemistry, AIIMS, Patna. This cross-sectional study included 100 consented healthy undergraduate medical and nursing students. **Methods and Material:** We isolated DNA from all saliva samples, amplified using multiplex PCR and gel electrophoresed to screen HPV 16 and 18. Feedback about the study in creating awareness regarding HPV and its vaccine was conducted using three-point Likert scale. **Statistical analysis:** The collected responses were entered in Microsoft excel. The results were expressed in frequency and percentages. **Results:** All saliva samples screened were found negative for HPV 16 and 18 DNA. Responses from feedback showed improved knowledge and awareness about the HPV and its vaccine among the participants. **Conclusion:** Even all the saliva samples tested were found negative for HPV DNA, the screening of high-risk HPV in saliva of young medical and nursing students generated curiosity among them to know more about HPV and its vaccine. This exercise may have helped in increasing the acceptance of HPV vaccine and the awareness of getting it at their ideal age to be benefited with dual protection, from oral and cervical (in case of females) cancers lifelong.

**Keywords:** HPV 16, HPV 18, human papilloma virus, oral cancer

## Introduction

Among all cancers prevalent in India, oral cancer ranks fifth in overall mortality.<sup>[1]</sup> The commonest of all, the squamous cell cancer accounts for 90–95% of oral cancer cases reported,<sup>[2]</sup> therefore needs to be taken care for early detection and prevention to reduce the disease burden. High risk HPV

type 16 and 18 has been linked to the causation of 25% of oral cancers.<sup>[3]</sup>

A published data from ongoing National Health and Nutrition Examination Survey study states that about 26 million Americans on any given day and seven in every 100 in United Kingdom have an oral HPV infection/cancer.<sup>[4,5]</sup>

Further, the same virus sub-types have also been reported to be responsible for 70% cervical cancer cases,<sup>[6]</sup> which may be attributed to the resemblance between cervical and oropharyngeal

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epithelium,<sup>[3]</sup> which is squamous cell type. Prevalence of oral HPV is on rise because of high-risk sexual behavior like oro-genital sex leading to sharing of viruses between oral and genital sites.<sup>[7]</sup> A study conducted in Brazil has reported HPV infections among sexually active adolescents and young adults.<sup>[8]</sup> Another study conducted on patients with oropharyngeal cancer from Middle East found a very high prevalence (85.3%) of HPV-related oropharyngeal cancer among them.<sup>[9]</sup>

Increasing prevalence among young adults and teenagers besides adults suggests other modes of transmission as well. It may be like near personal contacts with family members and through fomites at school and other educational settings.<sup>[10]</sup>

This study included medical undergraduate and the nursing students, who need to be aware of the possible modes of infection in the hospital settings. Diseases like recurrent respiratory papillomatosis, oral and anogenital warts, anogenital intraepithelial neoplasia are associated with HPV. Infective airborne particles are produced when these are treated by laser or electrosurgical procedures and therefore needs care.<sup>[11]</sup> Further, HPV 16 are resistant to glutaraldehyde and ortho-phthalaldehyde, the two disinfectants used in hospitals, and can even survive for months at low temperature without a host. Also the patients with planter warts harboring HPV can spread virus by walking barefoot.<sup>[12,13]</sup> Current evidence supports their transmission from mother to fetus during intrauterine period at the time of delivery and later through saliva.<sup>[14]</sup> These facts are important for medical and nursing graduates to be aware of.

With all epidemiological estimates, it is predicted that HPV positive oropharyngeal cancer cases will exceed the cervical cancers cases by the year 2020.<sup>[15]</sup> Therefore, vaccination against HPV 16 and 18 needs to be made mandatory, which may offer protection to both types of cancers, the cervical and oral. The HPV vaccination has also been recommended by CDC (Centers for Disease Control and Prevention) to get protection from cervical, oral, oropharyngeal, and anal cancers.<sup>[4]</sup> Since HPV infection is most prevalent in late teens and early 20s,<sup>[16]</sup> CDC suggests vaccination for everyone till the age 26 years if not vaccinated earlier.<sup>[17]</sup>

A study evaluating awareness and acceptability regarding HPV vaccine has shown that even among medical graduates, the desired knowledge lacks.<sup>[18]</sup> When incidence of oral cancer is increasing worldwide,<sup>[4,5]</sup> with high incidence rate among 15–24 years age group,<sup>[16]</sup> this study becomes highly relevant, keeping the awareness about HPV as priority among medical/nursing students who were young adults in their ideal age for vaccination, fulfilling our efforts to motivate them for the same.

## Subjects and Method

This cross-sectional study included 100 consented healthy undergraduate medical and nursing students and was approved by Institutional Ethical committee of AIIMS, Patna.

Inclusion criteria: Only consented healthy students were included. Exclusion criteria: Subjects on any medication were excluded. Approval from Institutional Ethical committee has been taken. Dated -06/12/2018.

## Interactive session

An interactive session with consented students was conducted prior to study. The HPV and its vaccine were discussed with the objective to increase the level of knowledge about the same.

## Saliva sample collection

The saliva was collected from the consented participants in a small, pre-labeled sterile collection tube with a buccal cell brush (Puregene Buccal Cell Core Kit A, QIAGEN). Victuals were not permitted 30 min preceding collection. The samples were stored at low temperature to reduce degradation of DNA. Each sample was allocated a distinctive and randomly generated number to forestall analysis bias. Demographic data relating to the sample was simultaneously collected. The samples were centrifuged at 4,000 g for 8 min at 4°C; the pellet was suspended in 10 mL of sterile normal saline buffer and stored at -80°C for DNA purification.

## DNA isolation

Genomic DNA was isolated from the stored samples using manufacturer's protocol with some minor modifications. DNA purity was calculated by ratio measurements of absorbance at 260 and 280 nm using Nano Drop One (Thermo Scientific, A260/A280 ratio between 1.8 and 2.20).

## Polymerase chain reaction (PCR)

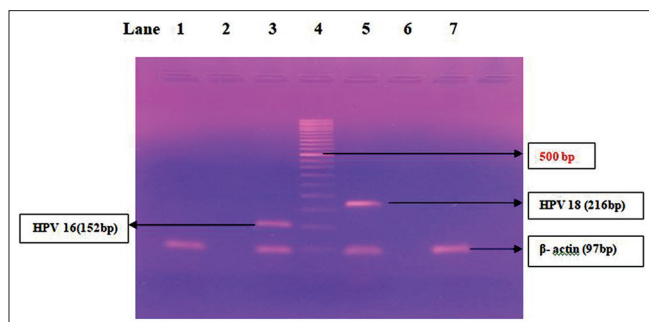
DNA from every isolated sample was used to carry out PCR to identify the respective HPV types using the primer sets given in Table 1.<sup>[19,20]</sup> The  $\beta$ -actin gene was used as an internal control. PCR reaction was optimized for this study using 2  $\mu$ g of DNA for each reaction. The reaction mixture had 1  $\times$  PCR Buffer, 1.5 mM MgCl<sub>2</sub>, and 0.4 mM of dNTP, 0.5  $\mu$ M of primer and 1U of Taq DNA polymerase. Amplification was performed using an Eppendorf thermal cycler following these steps; Initial denaturation at 95°C for 8 min, then 35 cycles of denaturation at 95°C for 45 s, primer annealing at 56°C for 30 s and extension at 72°C for 30 s; finally, an extension of 7 min at 72°C.<sup>[19]</sup>

## Results

The DNA isolated from the saliva samples (average concentration of 89.7 ng/ $\mu$ L) were checked for their purity using ratio measurements of absorbance at 260 and 280 nm. The average DNA purity was 1.84. The extracted DNA was amplified using multiplex PCR with type specific primers. All samples were screened using gel electrophoresis for the presence of HPV 16 and 18 sub-types. The human  $\beta$ -actin gene amplification was used as standard control. Multiplex PCR showed all the samples of students negative for HPV 16 and 18 [Figure 1].

**Table 1: Oligonucleotide Primer Sequences of HPV16, HPV18 and  $\beta$ -actin used for PCR**

Target Gene	Primers		Product size (bp)
	Forward	Reverse	
HPV 16	TGCTAGTGCTTATGCAGCAA	ATTTACTGCAACATTGGTAC	152
HPV 18	AAGGATGCTGCACCGGCTGA	CACGCACACGCTTGGCAGGT	216
$\beta$ -actin	CCAGAGGCGTACAGGGATAG	CCAACCGCGAGAAGATGA	97



**Figure 1:** Gel electrophoresis of PCR products. Lane 1: Student sample; Lane 2: Negative control; Lane 3: Positive control of HPV16; Lane 4: Molecular weight marker (50 bp); Lane 5: Positive control of HPV18; Lane 6: Negative control; and Lane 7: Student sample. Lower Panel:  $\beta$ -actin as an internal control

The mean age of the participants was 21.5 years (18–25 years). Almost all the participants were from good socioeconomic background having good oral hygiene and none were addicted to tobacco or alcohol.

### Qualitative analysis of the Feedback

Feedback about the study in creating awareness regarding HPV and its vaccine was conducted using three-point Likert scale.

Responses of the participants regarding their level of knowledge and awareness regarding HPV have been summarized in Figure 2.

About 60–70% of the students agreed that participation in this study has improved their knowledge about HPV, along with the awareness about the vaccine; its acceptance and recommendation [Figure 2]. However, 10–20% responses showed disagreement about the same whereas, 20–25% had a neutral response [Figure 2].

### Discussion

It is now evident that the HPV not only causes cervical cancer but is also a player in oral cancers, especially squamous cell carcinoma.<sup>[3]</sup> HPV positive OSCC (oral squamous cell carcinoma) have a better prognosis and menace to death is 60% of that of HPV negative OSCC, cause being strong loco regional control by HPV positive OSCC.<sup>[21]</sup>

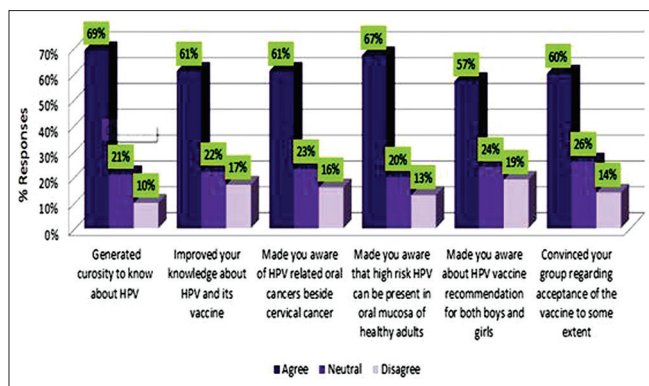
There has been a lack of knowledge among the young generation regarding HPV and its disease-causing potential. Poor socioeconomic status, a critical factor in maintaining body hygiene and oral health, increases the chance of HPV infection.<sup>[22]</sup>

Limiting tobacco/alcohol and maintaining a healthy diet may reduce the chances of HPV oral infections.<sup>[22]</sup> The development of vaccines against HPV has also raised hopes for prevention of cancers caused by it. However, poor awareness about the same, even among the medical students has been observed.<sup>[18]</sup> Our study has focused on screening of saliva of healthy young adults of age group 18–25 years, for HPV presence and creates awareness among these participants regarding HPV 16 and 18 sub-types in oral cancers including the benefits of vaccination which is recommended in this age group.

In our study, none of the saliva samples was found positive for HPV 16 or 18, which may be because of the fact that the study population belonged to an educated society with better socioeconomic status that had good oral hygiene and were not addicted to tobacco or alcohol. Also, the young population had an efficient immune system working for the protection from various infections. Similar results were found in previous study with 100 healthy subjects aged 20–31 years screened for HPV presence in saliva.<sup>[23]</sup> However, in other study, 6% HPV infected cases were detected in a mixed population of 268, which included subjects of less than 20 years of age.<sup>[24]</sup> This study showed that HPV was present primarily in the oral cavity of children of younger than 2 years of age and in adolescents of more than 13 years of age. This finding needs further explanation and suggests screening of saliva of the family members of the positive cases in the similar way.

Since our study had a small sample size (100 subjects) of participants of distinct group, reflecting sampling bias also, the results may not give the real picture about the HPV infection among the target population. However, this study definitely generated awareness among the participants as depicted in our results. The knowledge about the HPV and the outcome of infections associated with it have created the awareness among the study population of young adults, the need and the acceptance of vaccines to be taken at the right age. This may be one step in the right direction and also the need of the hour as one questionnaire based descriptive study has shown a very low acceptance rate (23%) of HPV vaccine in India.<sup>[25]</sup>

HPV vaccination has been implemented in the National immunization programs (NIPs) by more than 60 countries worldwide.<sup>[26]</sup> Those countries that have executed routine HPV vaccination in the recommended age group before 2010 have experienced a decrease in prevalence of high risk HPV type and diseases caused by them.<sup>[27]</sup> Safety of HPV vaccines has also been authenticated as no side effect has been reported even



**Figure 2:** Responses against the items of Feedback from the students. Responses of the participants regarding their level of knowledge and awareness regarding HPV

after giving 100 million doses worldwide.<sup>[27]</sup> High antibody titer against HPV-16 can predict the occurrence of HPV 16 related cancer about 10 years before it becomes obvious which may be a very useful approach for early diagnosis of such cancers.<sup>[14]</sup>

Dual dose of HPV vaccine at the age of 9–12 years for both boys and girls has been advocated by Advisory committee on immunization practices (ACIP).<sup>[28]</sup> Ideally HPV vaccine is recommended before possible exposure to HPV through sexual debut where it claims to provide maximum protection.<sup>[11]</sup>

Seeing the importance of vaccination, the United States, provides HPV vaccine free to the children belonging to low income groups under a federally funded program, Vaccine for Children (VFC).<sup>[29]</sup>

However, in India, the HPV vaccine is included under “The Indian Academy of Pediatrics Committee on Immunization” which recommends vaccination to only females aged 9–26 years.<sup>[30]</sup> In spite of this, a questionnaire based descriptive study has shown a very low acceptance rate (23%) of HPV vaccine in India, the root cause being lack of knowledge and high monetary cost of the vaccine.<sup>[25]</sup>

Seeing so much of awareness regarding HPV vaccine worldwide, its inclusion in NIPs by more than 60 countries and VFC program led this study to be conducted to benefit our medical students, for increasing their awareness regarding HPV immunization to receive in the ideal age to get dual protection from oral and cervical (among females) cancers in future. This study has relevance for the practices of primary care physicians, who have a role to play in providing comprehensive care to their patients. This care also includes protection from diseases including cervical and oral cancers. Such screening study would definitely be instrumental in fulfilling the role and responsibilities of primary healthcare system including specialists who render definitive care to the undifferentiated patient at the point of first contact, and continue to do so. In Indian settings, such type of study on young healthy adults has not been done so far to the best of our knowledge.

Small sample size is the limitation of the study and needs to be conducted on a much larger population. Further, study with large and different population groups will provide more awareness and firmer decision regarding inclusion and acceptance of HPV vaccination in the National Immunization Schedule for both boys and girls for prevention of oral carcinoma.

The highlight of this study is the approach with which the awareness about HPV16 and 18 and their role in causing oral and cervical cancer was created among young adults through a non-invasive screening with saliva.

The key points of this study are a) the non-invasive method of screening for the presence of HPV 16 and 18 b) the selected group of subjects in the study, the young adults who need to get aware for timely protection from the disease caused by the virus: and last but not the least c) the study may help to incorporate immunization against high risk HPVs in health care decision-making to reduce the disease burden in the future. A novel message conveyed through this study is that there is a breach in the knowledge regarding HPV related oral cancer in India and also for its vaccine which necessitate this study to be conducted.

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## Conflicts of interest

There are no conflicts of interest.

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