

## RESEARCH COMMUNICATION

# Prevalence and Distribution of High Risk Human Papillomavirus (HPV) Types 16 and 18 in Carcinoma of Cervix, Saliva of Patients with Oral Squamous Cell Carcinoma and in the General Population in Karnataka, India

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### Abstract

**Background:** In view of conducting HPV vaccination in India it is most important to understand the prevalence of HPV genotypes in this population, not only in squamous cell carcinoma of cervix and oral cavity but also in the general population. In this study we explored the prevalence and distribution of high-risk HPV types 16 and 18 in carcinoma of cervix, saliva of patients with oral squamous cell carcinoma and in general population in Karnataka. **Methods:** Cervical cancer specimens after punch biopsy (n=60) were obtained from women attending Karnataka Institute of Medical Sciences and Karnataka Cancer Therapy and Research Institute, Hubli (KCTRI). Saliva rinse of (n=34) OSCC patients from KCTRI and (n=396) normal individuals from different regions of North Karnataka, were collected and PCR based high-risk HPV genotyping was carried out. **Results:** Using consensus PCR primers it was observed that 96.7% patients were infected with HPV irrespective of specific type in cervical cancer. Among them, HPV 16 was observed in 89.7%, HPV 18 in 86.2% and both HPV 16 and 18 in 79.3% patients. In OSCC, 70.6% were positive for HPV, among which HPV 16 prevalence was observed in 45.8%, HPV 18 in 54.2%, and HPV 16 and 18 multiple infection in 4.18%. In general population, HPV prevalence was observed in 84.4%. Among them, HPV 16 was observed in 2.75% and HPV 18 in 22.0% patients. In general population, multiple infection with HPV 16 and 18 was not observed but 75.3% were found to be infected by HPV genotypes other than HPV 16 & 18. **Conclusions:** Our study reveals that multiple infection of HPV 16 and 18 is quite high in cervical cancer and in case of OSCC, it was in conformity with the other studies. In general population HPV 18 prevalence was observed to be high. With this, we can conclude that both HPV 16 and 18 vaccinations will reduce the burden of cervical cancer and OSCC in Karnataka.

**Keywords:** Cervical cancer - oral SCC - HPV - high risk types - Karnataka, India

*Asian Pacific J Cancer Prev*, 12, 645-648

### Introduction

HPV being one of the most common STIs (Bosch et al., 1995), has been established as the main cause of cervical cancer. In developing countries cervical cancer is the leading cause of cancer death in women (Syrjanen et al., 1990; Koutsky, 1997). According to the ICMR report cervical cancer is the prime cause of deaths among women in India. Approximately 20,000 new cases were detected in India, in the year 2000 (Sankaranaryanan et al., 2001). Presently, in India, screening for cervical cancer is carried out cytologically (Pap test). Due to poor health services and high cost involved in the test, it is practically not possible to offer this to large female population of our country. In spite of all these facts, it is clear from large case studies, that HPV infection is the major risk factor for

the development of cervical intraepithelial neoplasia and that, risk is significantly increased by persistent infection with high-risk or cancer associated genotypes (Deacon et al., 2000; Franceschi et al., 2003; Diane et al., 2004; Cuschieri, et al., 2005).

Oral squamous cell carcinoma (OSCC) accounts for over 90% of oral cancers (Luo et al., 2007; Ajay Kumar Chaudhary et al., 2010). It has been established that smoking, heavy alcohol and betel nut chewing are the etiological factors for OSCC. However increasing research and technology have focused on identifying possible viral etiological factors such as oncogenic human papilloma virus (Ringström et al., 2002). Absence of well defined early symptoms is one of the major factors in lack of diagnosis for OSCC not only in India but also elsewhere. Hence, development of specific biomarker is

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likely to be important in screening of high-risk patients (Li et al., 2004). Till date no reliable or clinically applicable biomarker has been identified for testing in large population. Therefore, development of saliva based screening test for OSCC maybe able to detect early stage tumors before the development of clinical features (SahebJamee et al., 2008). The DNA based direct detection of HPV types may offer an alternative or complement the population-based cytological screening. The aim of this screening was to detect HPV in saliva of patients with OSCC and in general population to evaluate the possible high risk HPV infection.

In this study, we carried out PCR based high risk HPV genotypes 16 & 18 screening in cervical cancer, OSCC and general population using same HPV consensus primers and HPV type 16 & 18 primers.

## Materials and Methods

### Collection of cervical cancer specimens:

All the 60 patients included in this study were admitted and being treated at Karnataka Institute of Medical Sciences (K.I.M.S.) and Karnataka Cancer Therapy and Research Institute (K.C.T.R.I.), Hubli, Karnataka, India between 2009 & 2010. After confirming cervical cancer histopathologically, patients were asked to participate in the current study and patients who were comfortable with this study were recruited, after taking consent in vernacular. A 5m.m. tissue by punch biopsy was collected from each patients and was stored in RNA later in -80° C until further use.

### Collection of saliva rinse:

Histopathologically confirmed 34 OSCC patient's saliva was collected in sterile 15mL falguen tubes after taking consent of the patients in vernacular from Sri Dharmasthala Manjunatheshwara (S.D.M.), Dental College, Dharwad, India. 396 saliva samples from normal individuals were also collected randomly from rural and urban (8 villages & 3 taluks) areas, after explaining the purpose of the study.

Irrespective of age & sex the samples were collected randomly and high-risk HPV genotyping was carried out by PCR based method at Research center for DNA Diagnostics, Karnatak University, Dharwad.

### Genomic DNA isolation:

Genomic DNA from cervical cancer tissues was isolated by commercially available kit (QIAGEN, USA) as per manufacturer's instructions. DNA from saliva was isolated by standardized protocol as follows: the samples were centrifuged at 4000rpm for 2min. The supernatant was collected and 250µl of 10% SDS was added and mixed well and 5µl of Proteinase K and RNase H each were added then allowed to stand at room temperature for 45-60 min. To this 150µl of Phenol:Chloroform:Isoamyl alcohol (25:24:1) was added and mixed well, centrifuged at 4000rpm for 5min. Supernatant was collected into 2ml sterile microcentrifuge tubes and chilled absolute alcohol (3 volumes) was added, and centrifuged at 13000rpm for 5 min. DNA pellet was collected, dried and dissolved in

150µl of T50E20 solution. All the DNA samples were confirmed on 0.8% agarose gel and quantified using biophotometer (Eppendorf, Germany).

### Polymerase Chain Reaction:

HPV consensus, 16 & 18 specific primers were obtained as described in earlier study (Ramdas Chatterjee, et al., 2005). Initially all the samples were screened by HPV consensus primers to select the HPV positive specimens and only the specimens which were positive were tested for HPV 16 & 18 specific types. Genomic DNA isolated from HeLa cell lines were used as positive control in all the PCR reactions. PCR amplification was carried out in a 20µl reaction volume containing 0.5µl of genomic DNA (75ng/µl to 150ng/µl), 0.5µl of each primers (5p.mol.), 0.4µl of dNTP (10p.mol.), 0.2µl of Taq DNA polymerases (3units/µl) along with Taq buffer 4.0µl(Bangalore GeNei, India) and the total volume was adjusted to 20.0µl using molecular biology grade water. Amplification was carried out in Mastercycler gradient (Eppendorf, Germany) under the following conditions. An initial denaturation at 98°C for 10sec, followed by 35 cycles at 98°C for 10sec (cycle denaturation), primer annealing temperature was set depending on the annealing temperature of each primer (14) for 10sec, 72°C for 15sec (primer extension), and a final extension of 72°C for 5min. PCR products were confirmed for their respective amplicon size by gel electrophoresis with standard 100bp ladder.

## Results

Sixty patients with cervical cancer, thirty four patients with OSCC and 396 normal individuals were included in this study. Out of them, 58 cervical cancer patients, 24 OSCC patients and 255 individuals from normal population were positive for HPV infection.

Table 1 represents the prevalence and distribution of high-risk HPV types in cervical cancer tissues: Out of 58 HPV positive tissue sample, HPV 16 was observed in 52 (89.66%) samples, HPV 18 was observed in 50 (86.215%) samples. HPV 16 & 18 together were identified in 46 (79.31%) samples.

## Discussion

Increasing cases of cancers associated with HPV infection call for a vaccination programme or other treatment measures across India. In order to develop such measures we first need to evaluate the prevalence of HPV high risk genotypes in different parts of India. Therefore,

**Table 1. Prevalence of HPV Types in Cervical Cancer Patients**

	Number	Percentage Total
Total Sample Size	60	
HPV Positives	58	96.7%
HPV Negatives	02	3.33%
HPV 16	52	41.7%
HPV 18	50	54.2%
HPV 16 & 18	46	4.17%

**Table 2. Prevalence of HPV Types in OSCC Patients**

	Number	Percentage Total
Total Sample Size	34	
HPV Positives	24	70.6%
HPV Negatives	10	29.4%
HPV Types		
16	10	41.7%
18	13	54.2%
16 & 18	1	4.17%

**Table 3. Prevalence of HPV Types in General Population**

	Number	Percentage Total
Total Sample Size	396	
HPV Positives	255	64.4%
HPV Negatives	141	35.6%
Types		
HPV 16	7	2.75%
HPV 18	56	22.0%
HPV 16 & 18	192	75.3%

in this study we have evaluated the prevalence of high risk HPV types 16 & 18 among cervical cancer patients (n=60), OSCC patients (n=34) and normal individuals (n=396) to determine the risk of cancer development in the population due to HPV infection.

Clinically certified cervical cancer tissues, saliva samples of OSCC patients and saliva samples of general population was taken for PCR based analysis of HPV infection. HPV prevalence was observed to be 96.67% (n=58/60) in case of cervical cancer tissues among which prevalence of HPV 16 (89.66%, 52/58) was observed to be higher than HPV 18 (86.21%, 50/58). In case of OSCC patients, 70.59% (n=24/34) HPV prevalence was observed, among which HPV 18 infection (45.83%,

11/24) was observed to be higher than HPV 16 (54.17%, 13/24). In general population, 84.39% (n=255/396) HPV prevalence was observed with a higher HPV 18 prevalence (2.75%, 7/255) than HPV 16 (21.98%, 56/255).

Our evaluation showed a slightly higher HPV prevalence in cervical cancer tissues compared with saliva samples of OSCC and general population. Higher cases of multiple infections of HPV 16 & 18 were also seen in case of cervical cancer in comparison with OSCC and general population.

In general population of Karnataka, HPV 18 genotype was found to be most frequently distributed compared to HPV 16 genotype which has higher frequency as observed in the study by A Pavani, et al., 2005, in Andhra population (Table 4).

As tabulated in Table 5, according to studies conducted globally, prevalence of HPV genotypes among general population ranges between 20-40%, with HPV 16 prevalence ranging between 3-17% and HPV 18 being 0-14%; HPV prevalence among cancer patients ranging from 35-91%, prevalence of HPV 16 between 27-54% and HPV 18 prevalence ranging between 5-14%. In comparison with these observations our study results show deviation in the HPV prevalence pattern for the general population. HPV 16 is observed to be more prevalent than HPV 18, which is contrary to the findings of the studies conducted by Cosette et al., 2009, at U.S.A.; Polat et al., 2009, at Turkey; Cuschieri et al., 2004, at U.K.; and Mahnaz et al., 2009 at Iran.

In case of cervical cancer, our results are in conformity with the results of the study by A Pavani et al., 2005, at Andhra Pradesh, India and Cosette et al., 2009, at U.S.A. which show 87.8% and 91% HPV prevalence respectively.

**Table 4. Comparison of HPV Prevalence in Cervical Cancer in India**

Region of Study	Sample Type	HPV Genotype	Percentage Prevalence	Authors
Andhra Pradesh	General population	HPV all types	10.3%	Pavani Sowjanya, et al.
		16	17.6%	
		18	5.9%	
	Cervical Cancer Patients	HPV all types	87.8%	
		16	66.7%	
		18	19.4%	
Karnataka	General Population	HPV all types	64.3%	Present study
		16	2.75%	
		18	1.96%	
	Cervical Cancer Patients	HPV all types	96.7%	
		16	89.7%	
		18	86.2%	

**Table 5. Comparison of HPV Prevalence in Cancer Globally**

Region of study	Sample type	HPV Genotypes % Prevalence			Authors
		HPV all types	HPV 16	HPV 18	
U.S.A.	General population	38.4%	7.4%	1.1%	Closet et al.
	Cervical cancer patients	91.0%	53.2%	13.1%	
Turkey	General population	20%	37%	14%	Dursun et al.
	Cervical cancer patients	36%	30%	5%	
Edinburgh, U.K.	General population	20%	3.4%	1.4%	Cuschieri et al.
	Cervical cancer patients	46%	48.9%	11.7%	
Iran	General population	25%	20%	0%	Saheb Jamee et al.
	OSCC patients	40.9%	27.3%	4.55%	
Karnataka, India	General population	64.29%	2.75%	21.96%	Present study
	Cervical cancer patients	96.67%	89.66%	86.21%	
	OSCC patients	70.59%	41.67%	21.96%	

The studies at Turkey and U.K. show lesser prevalence of HPV. The prevalence of HPV 16 is observed to be higher than HPV 18 which is in agreement with the studies conducted globally.

In case of OSCC it is observed that in our study the prevalence of both HPV 16 and HPV 18 is quite higher than what was observed in the study conducted by Mahnaz Saheb et al., 2009, at Iran.

From these observations it is evident that, there is quite a high percentage of HPV prevalence even in the general population of Karnataka in comparison with the studies conducted in India as well as studies of global HPV prevalence. The differential distribution of HPV genotypes in different regions of India could provide a better perspective for the development of appropriate HPV vaccination programme in India.

In conclusions, Our results confirm the infection of HPV high risk genotypes 16 & 18 as major factor in the development of Cervical and Oral squamous cell carcinomas in the population of Karnataka. The high prevalence of HPV genotypes in general population suggests towards vaccination for HPV genotypes as an essential measure for reducing cancer risk due to HPV infection.

## Acknowledgments

The authors are thankful to the Department of Medical and Higher Education Govt. of Karnataka for providing financial assistance to undertake this work. We are grateful to the doctors and the staff of KCTRI, Hubli; S.D.M., Dental College, Dharwad and K.I.M.S., Hubli for co-operating with us in the sample collection from cervical cancer and OSCC patients. We are also thankful to the local Taluk Panchayats for allowing us to enroll general population from various regions in this study.

## References

Bosch FX, Manos MM, Munoz N, et al (1995). Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) study group. *J Natl Cancer Inst*, **87**, 796-802.

Chaudhary AK, Pandya S, Mehrotra R, et al (2010). Comparative study between the Hybrid Capture II test and PCR based assay for the detection of human papillomavirus DNA in oral submucous fibrosis and oral squamous cell carcinoma. *Viral J*, **7**, 253.

Cuschieri KS, Cubie HA, Whitley MW, et al (2004). Multiple high risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. *J Clin Pathol*, **57**, 68-72.

Cuschieri KS, Cubie HA, Whitley MW, et al (2005). Persistent high risk HPV infection associated with development of cervical neoplasia in a prospective population study. *J Clin Pathol*, **58**, 946-50.

Deacon JM, Evans CD, Yule R, et al (2000). Sexual behaviour and smoking as determinants of cervical HPV infection and of CIN3 among those infected: a case-control study nested within the Manchester cohort. *Br J Cancer*, **88**, 1565-72.

Dursun P, Senger SS, Arslan H, et al (2009). Human papillomavirus (HPV) prevalence and types among Turkish women at a gynecology outpatient unit. *BMC Infect Dis*,

**9**, 191.

Franceschi S, Rajkumar T, Vaccarella S, et al (2003). Human papillomavirus and risk factors for cervical cancer in Chennai, India: a case-control study. *Int J Cancer*, **107**, 127-33.

Harper DM, Franco EL, Wheeler C, et al (2004). Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *Lancet*, **364**, 1757-65.

Koutsky L (1997). Epidemiology of genital human papillomavirus infection. *Am J Med*, **102**, 3-8.

Li Y, St John MA, Zhou X, et al (2004). Salivary transcriptome diagnostics for oral cancer detection. *Clin Cancer Res*, **10**, 8442-50.

Luo CW, Roan CH, Liu CJ (2007). Human papillomaviruses in oral squamous cell carcinoma and pre-cancerous lesions detected by PCRbased gene-chip array. *Int J Oral Maxillofac Surg*, **36**, 153-8.

Ramdas Chatterjee, Biplab Mandal and Sarmistha Bandyopadhyay. Detection of HPV DNA in Cervical Carcinomas after Treatment in India. *Int J Hum Genet* 2005, **5**(1): 27-31.

Ringström E, Peters E, Hasegawa M, et al (2002). Human papillomavirus type 16 and squamous cell carcinoma of the head and neck. *Clin Cancer Res*, **8**, 3187-92.

SahebJamee M, Boorghani M, Ghaffari SR, et al (2009). Human papillomavirus in saliva of patients with oral squamous cell carcinoma. *Med Oral Patol Oral Cir Bucal*, **14**, 525-8.

SahebJamee M, Eslami M, AtarbashiMoghadam F, et al (2008). Salivary concentration of TNFalpha, IL1 alpha, IL6, and IL8 in oral squamous cell carcinoma. *Med Oral Patol Oral Cir Bucal*, **13**, E292-5.

Sankaranarayanan R, Budukh AM, Rajkumar R (2001). Effective Screening Programmes for Cervical Cancer in low- and middle-income developing countries. *Bull World Hlth Organ*, **79**, 954-62.

Sowjanya AP, Jain M, Poli UR, et al (2005). Prevalence and distribution of high-risk human papilloma virus (HPV) types in invasive squamous cell carcinoma of the cervix and in normal women in Andhra Pradesh, India. *BMC Infect Dis*, **5**, 116

Syrjanen K, Yliskoski M, Kataja V, et al (1990). Prevalence, incidence, and estimated life-time risk of cervical human papillomavirus infections in a nonselected Finnish female population. *Sex Transm Dis*, **17**, 15-9.

Wheeler CM, Hunt WC, Joste NE, et al (2009). Human papillomavirus genotype distributions: implications for vaccination and cancer screening in the United States. *J Natl Cancer Inst*, **101**, 475-87.