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Original Research Article

Prevalence of High-Risk Human Papilloma Virus (HR-HPV) As a Risk Factor in HNSCC Patients of Saurashtra Region of Gujarat

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ABSTRACT

Background: In India HNSCC comprises the largest group of malignancies with an incidence rate as high as 30-40%.The present study was carried out to determine the prevalence of high-risk human papilloma virus (HR-HPV) as a risk factor in HNSCC patients of Saurashtra region of Gujarat.
Method: Newly diagnosed 200 HNSCC patients were selected for the study. Sociodemographic and clinical data were obtained through questionnaire. Detection of HPV-DNA was done from cancer tissues by PCR amplification method using GP5+/GP6+ primers, E6 and E7 primers for HPV 16 and HPV 18 genotypes.
Result: The prevalence of HPV high-risk (HR) types was 2% in HNSCC cancer cases. HPV 16 genotype was identified while HPV 18 was absent in all the patients. The risk factor of HPV-HR included younger age (<55 years) and early age at first sexual intercourse. The other risk factors like tobacco and alcohol were absent in these patients. The site of cancer was found to be base of tongue and tonsil.
Conclusion: The specific characteristics found in HPV positive HNSCC cases are in accord with distinctive characteristics of HPV positive HNSCC found worldwide. We can conclude that HR-HPV infection may be responsible for HPV-positive HNSCC. However, the prevalence of HPV among HNSCC is negligible which indicates that HPV is not an influential risk factor for oral cancer in this region.

Key Words: HR-HPV, Head and Neck squamous cell carcinoma, PCR.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCCs) represent the sixth most common malignancy and a significant cause of mortality worldwide. In India HNSCC comprises one of the largest group of malignancies with an incidence rate as high as 30-40%. Within the country the spectrum of HNSCC varies from place to place with a striking increase in the incidence in states like Uttar Pradesh, Madhya Pradesh, Gujarat, Bihar and Maharashtra. [1]

The major established etiological factors are smoking and alcohol. There appears 5-fold to 25-fold higher risk of developing head and neck cancers in heavy tobacco users than in nonsmokers. [2] Human Papilloma Virus which is the causative agent for cervical cancer is now documented to have a role in the pathogenesis of head and neck squamous cell carcinoma (HNSCC). [3,4] Overall incidence of HNSCC has fallen in the last three decades; however the incidence of oropharyngeal carcinoma, mainly tonsil and

base of tongue, has been increasing both in United States and Europe. [5,6] Approximately 25% of HNSCC are reported to have association with HPV and about 60% of oropharyngeal cancers are found to be associated with HPV. The most susceptible sites for HPV infection are the oral cavity, pharynx and larynx, epithelial cells. [7-9]

Over the last decade the significant rise in the cases of HPV-positive Oropharyngeal squamous cell carcinoma (OPSCC) has been reported among nonsmoking, young patients. [10,11] This indicates oral HPV infection as a causative agent, possibly due to changes in sexual behavior. [12] Various studies have reported a varying degree of incidence of HPV infection in different population from different geographical regions. In the span of 16 years between 1988 and 2004, there was a 225% rise in the occurrence of HPV-positive cases of OPSCC, and a concomitant 50% fall in HPV-negative OPSCC in the United States [10] with similar trends reported in Europe and Australia. [13, 14]

HPV-16 is the most common HPV in HNSCC established in 90–95% of all HPV positive HNSCC cases, followed by HPV-18, HPV- 31 and HPV-33. [15,16] The oncogenic ability of the High Risk HPV (hr-HPV) types 16 and 18 is through the expression of E6 and E7 oncoprotein which inactivates TP53 and retinoblastoma tumor suppressor gene respectively. [3,17]

Variable results regarding association of HPV with HNSCC has been reported in different regions of India which may be due to the heterogeneity of the patient populations, different risk factors and changing life styles. The occurrence of HPV in HNSCC was found to be 15% in western India, [18] 33% in East India, [19] 32% in north India [20] and 48% in south India. [21]

Saurashtra region of Gujarat is well known for harboring large number of HNSCC cancer cases. However the association of HPV with HNSCC has not been investigated in this region till date. Hence, the present study is aimed to learn the incidence and prevalence of HPV in HNSCC patients.

MATERIALS AND METHODS

Patients:

The patients who were histologically diagnosed to have squamous cell carcinoma of head and neck were included in the study. The study was approved by Ethical Committee of Rajkot Cancer Hospital. The patients gave the informed consent for the study. The patient information was obtained through questionnaire that included demographic (age, gender, marital status, education, occupation, geographic location) and clinical data (family history of cancer, tobacco and alcohol consumption, nutritional status). The information of signs and symptoms of their oral lesion, its duration were also taken. The questionnaire also included the questions regarding life time number of sexual partners, age at first intercourse and number of oral-genital partners.

Samples:

The malignant tissue samples from the oral lesion were collected at the time of surgery. They are snap frozen in liquid nitrogen and stored at -80^{o}c until further use.

DNA extraction

DNA was isolated from the tumor tissues by modified Phenol-chloroform method. Briefly about 10-15 mg of tissue was ground into fine powder by mortar and pestle. The powdered tissue was then suspended into nucleic lysis buffer for 3 hrs at 56^{o}c. The phenol: choloroform method was used to extract the DNA and K-acetate was used to salt out the DNA. The DNA was then precipitated by isopropanol and suspended in TE buffer. Spectrophotometric analysis of isolated DNA was done to check the purity of the DNA. This DNA was used for further analysis in polymerase chain reaction.

HPV detection and typing

Amplification of beta globin, a house-keeping gene was carried out using

human genomic DNA isolated from all the clinical samples to check the presence of amplifiable DNA.

All beta globin positive genomic DNA samples were then subjected to PCR to detect the presence of HPV DNA. Consensus primers GP5+/GP6+ were used to detect HPV from wide range of HPV genotypes including low risk and high risk types. Only those samples found positive for HPV were selected for further specific genotyping. The consensus primers from the L1 region of high risk HPV 16 and 18 were used for PCR assay for definite HPV genotype identification.

PCR amplification was performed in 25 µl reaction volume that contained 4µl of genomic DNA, 1 µl of each primers, 2µl of dNTPs, 2 µl of $MgCl_2$, 0.6 µl of Taq DNA polymerases (3units/µl), along with Taq buffer 2.6µl. The PCR conditions for GP5+/ GP6+ were as follows: initial denaturing step- 95°C for 5 min, followed by 35 cycles of 95°C for 45 seconds, annealing step of 55°C for 45 seconds and extension step of 72°C for 45 seconds. This was followed by a final extension period of 7 min at 72°C. The amplification was performed in eppendorf mastercycler. The primer sequences used are shown in Table 1.

The PCR products were then analyzed through gel electrophoresis with standard 100bp ladder and HPV-16 and HPV-18 positive control (Acrometrix) and negative control.

Table 1: Primer sequences

Primer	Sequence	Product size in bp
PCO4	5'-CAACTTCATCCACGTTCACC-3'	268
GH2O	5'-GAAGAGCCAAGGACAGGTAC-3'	
HPV GP5+	5'-TTT GTT ACT GTG GTA GAT ACT AC -3'	285
HPV GP6+	5'-GAA AAA TAA ACT GTA AAT CAT ATT C-3'	
HPV 16 L1 F	5'-TGCTAGTGTTATGCAGCAA-3'	150
HPV 16 L1 R	5'-ATTTACTGCAACATTGGTAC-3'	

RESULTS

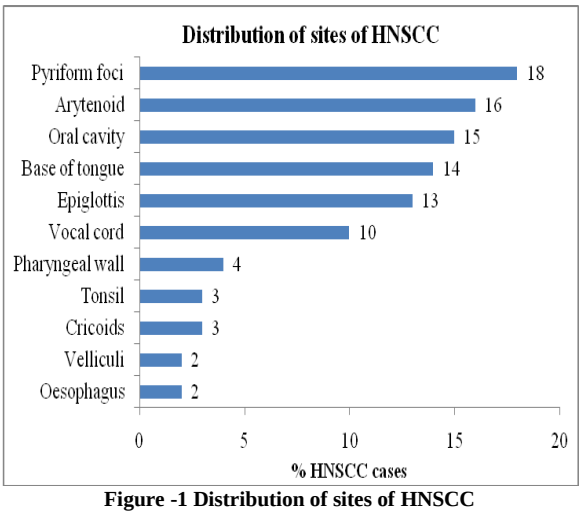

Figure -1 Distribution of sites of HNSCC

For this study, 200 patients were included for detection of HPV in HNSCC with 80% male and 20% female patients. The mean age was 46 years. The site distribution showed the predominance of hypopharynx followed by oral cavity and tonsil (Figure-1). Among the risk factors for HNSCC patients in Saurashtra region in the present study, tobacco smoking ranked the first (57 %), followed by tobacco chewing (20%) and alcohol drinking (4%). The 19 % of patients were nonsmokers and nondrinkers (Figure-2).

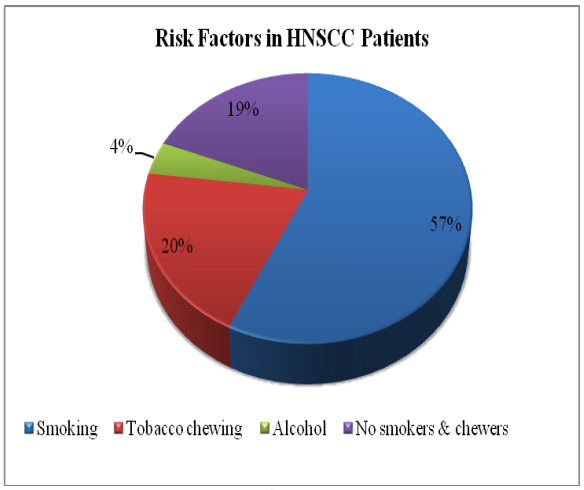

Figure-2 Risk factors in HNSCC

Only two patients (1%) were found to have positive results for HPV (Figure-3). Both the patients showed the presence of HPV 16. HPV 18 was absent in both the patients. The sites of cancer in these two patients

were base of tongue and tonsil respectively. None of the other sites of cancer showed the presence of HPV. These HPV positive patients were young at the time of diagnosis. The one of the risk factors associated with these HPV positive cases was the history of sexual activity at very young age. However, the patients denied having multiple sex partners. The other risk factors like tobacco smoking or chewing and alcohol drinking were absent in these cases.

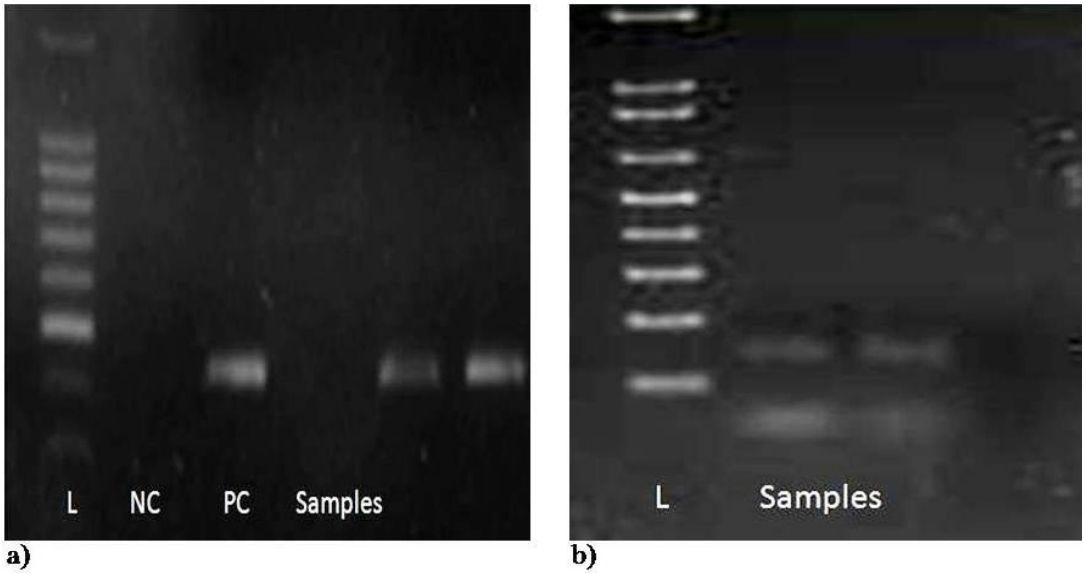

Figure -3 (a) HPV Consensus primers GP 5+/GP 6+, 285bp, (b) HPV 16 L1 primers, 150bp L-100bp Ladder, NC-Negative control, PC- Positive control

DISCUSSION

The epidemiology of HNSCC has dramatically changed over the past two decades. The overall incidence of HNSCC has decreased in the developed countries in last decade as result of a decline in the consumption of tobacco. However, there is a simultaneous increase in the incidence of OPSCC due to Human Papilloma Virus (HPV) infection. In the Western countries about 60% of OPSCC patients are found to have infection with the oncogenic forms of HPV, particularly HPV-16. [22-24] Worldwide 25.6% of OPSCC cases have been estimated to be HPV-related and geographical variations of cases of HPV-positive OPSCC have been found. The percentage of HPV-positive OPSCC cases was 56% in North America; 52% in Japan; 45% in Australia; 39% in Northern and Western Europe; 38% in Eastern Europe; 17% in Southern Europe; and 13% in the rest of the world. [25]

The prevalence of HPV in HNSCC across India has shown a wide variation. In Western India HPV incidence was found to be 0%. [26] A study reported the presence of high risk HPV 16 and HPV 18 in 6% of the patients of Mumbai, Maharashtra. [27] In North Indian population the prevalence of HPV was reported to be 32.4%. [28] Study in South India reported 74% HPV occurrence in oral cancer patients with 42% and 47% cases of HPV16 and HPV18. [29]

The present study of association between HPV and Head and neck cancer is the first study in Saurashtra region of Gujarat. Our study reported the 1% occurrence of High Risk HPV in HNSCC. Our results are in agreement with studies done in Gujarat and Mumbai in West India, and Kerala in South India which have reported a null or negligible prevalence of HPV in oral cancer. [26, 27, 30]

The present study showed the presence of HPV in tumors with oropharynx as the site of cancer. This observation is consistent with data that showed HPV is found to be highly prevalent in cancer of

oropharyngeal region that include the base of tongue and tonsils. [16, 31]

The results in this study showed the presence of HPV-HR types in young patients of HNSCC, which is consistent with the studies that reported 36–78% positivity in those less than age 50–60 years compared to 12–29% in those greater than age 60. [32] Patients who have HPV-associated OPSCC are less likely to have any habit of tobacco of alcohol use as compared to tobacco-related HNSCC. HPV positive HNSCC patients in our study were nonsmoker and nondrinker of alcohol. This is in agreement with observation of Klussman JP et al and Smith et al which have found that HPV-HR carcinoma was found frequently in patients with lower alcohol and/or tobacco exposures. [16,33] Nevertheless, more number of oropharyngeal cancer cases may be studied for HPV detection to ascertain its role and prevalence in Saurashtra region.

CONCLUSION

The specific characteristics found in our HPV positive HNSCC cases are in accord with distinctive characteristics of HPV positive HNSCC found worldwide. Hence we can conclude that HR-HPV infection may be responsible for HPV-positive HNSCC in these cases. However, we found negligible incidence of high risk HPV in HNSCC patients in Saurashtra. This indicates that HPV is not a major risk factor of occurrence of HNSCC in this region. The risk factors like smoking and chewing tobacco are still the most common causative agents of oral cancer in this region.

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