Human papilloma virus in head and neck carcinoma: Experience from a regional cancer center in Gujarat.

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Abstract

Introduction
We investigated the etiological role of HPVs in patients with head and neck cancers and aimed to study its detection tumor tissues taken cancer patients. Methods: 100 patients with malignancies of buccal mucosa, tongue and right maxilla were included in the study. Tumor biopsy was taken for histopathology and molecular studies for by multiplex PCR.

Results
Out of 100 head and neck cancers, 81 % were males and 19 % females, 69% belonged to age >50 yrs and 31 % were < 50 yrs, 58 % were from urban area. Tobacco chewing is one of the contributory factors in the genesis of oral carcinomas and we noted that 35% were non-chewers and 65 % chewers. 61% were smokers and 39 % non-smokers. HPV genome was detected in 20% of the cases. High risk HPV-16 was detected in 35% and HPV 52 in 20%. Out of the 43 biopsies from buccal mucosa 7 cases had HPV, from 20 cases of carcinomas tongue 9 cases had HPV positive(40%), one case of carcinoma thyroid had HPV positive, 3 cases of carcinoma maxilla had HPV positive. The oral cavity tumors like the buccal mucosa and tongue significantly had more HPV positive as compared to oropharynx. Other HPV types detected were HPV-33(15%), HPV-39(5%), HPV-45(10%), HPV-52(20%) and HPV-58(15%).

Conclusion
The SCC and Head and Neck had 20% of HPV types. Different HPV types were HPV16, 33, 39, 45, 52 and 58. Though the biopsies were from carcinomas, our study did not show that there is 100% co-relation of HPV as a sole etiological agent in the genesis of the squamous cell carcinoma. Oral cancers occurred typically in > 50yrs of age and, old heavy drinkers and heavy smokers.

Introduction
Head and Neck cancer attribute to be the 5th most common malignancy reported globally and it has been represented as heterogeneous neoplasias. Majority of them are squamous cell carcinomas originating from epithelium. Oral cancers occur typically in 60-65 yrs old heavy drinkers and heavy smokers. Some studies suggest that 15% - 25% of the oropharyngeal cancers have HPV-16. Epidemiological and molecular data have indicated the role of High Risk HPV genotype in these cancers. Tobacco and alcohol consumption are the 2 major risk factors for such cancers. High risk HPVs are etiological agents for anogenital tract cancers and have been detected in head and neck cancers. Epidemiological and experimental evidences has implicated oncogenic HPV causing subset of head and neck cancers, which has strongly supported the recent large case control study by the “International agency for research on cancer” (IARC). In all studies, the association of HPV exceeds 50% in tonsils and 90% were HPV-16 and are defined subset of head and neck cancers like oropharynx, tonsils and throat and have markedly improved prognosis. Two viral oncoproteins of high risk HPVs, E6 and E7 promote tumor progression. Accordingly, these viral oncoproteins are capable of transforming primary human keratinocytes disrupting cell cycle regulatory pathways in the genetic progression of squamous cell carcinoma (SCC). We investigated for the etiological role of HPVs in patients with head and neck cancers and aimed to study its detection in tumor tissues.

Materials & Methods

Subjects
Between August 2009 to January 2011, a total of 100 patients clinically suspected as Head and neck cancers were subjected for biopsy and sent for histological and molecular studies. The procedures followed were in accordance with the ethical standard and approved by institute ethical committee. Patients’ written consent was taken and detailed history of the patient was recorded. Demographic data including age, sex, and consumption of alcohol and tobacco exposure were obtained from the patients. Patients were classified as non-alcohol drinkers, alcohol drinkers, as smokers and non-smokers, tobacco chewers and non-chewers. Tumor site and histological type was recorded.

Tumor specimens
Two fresh tumor specimens were collected from different sites of upper respiratory tract like oral cavity, oropharynx, and hypopharynx in sterile normal saline for molecular studies and in formalin for histopathology. Tissue in saline was stored at -20°C until processed and tissue in formalin was processed for haematoxylin and eoslir staining for histopathology diagnosis. Tumor type was recorded. Approximately 25 mg of tissue was taken from the tumor specimen for the extraction of the HPV DNA and placed in a 1.5 ml micro centrifuge tube and 300 ul lyses solution was added. Then vortexed it and incubated for 5 mins at 650C and then centrifuged for 7-10 seconds. 20 ul of sorbent was added to each tube, incubated tube for 3 mins at room temperature. Then centrifuged tubes for 30 secs at 5000 g.

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Then supernatant was discarded without disturbing the pellet. 500ul of washing solution was added to the tubes and centrifuged for 30 seconds at 10,000 g. Again supernatant was removed. Then incubated for 5 mins. at 650C and vortexed periodically. Pellet was resuspended with 100 ul of DNA elution buffer. Later incubated for 5 min at 65°C and centrifuged for 1 min at 12000 g. And finally supernatant containing the desired DNA was available for amplification. Extracted DNA was stored at -20°C until processed.

Multiplex PCR kit (Saccase kit) which contains PCR master mix, buffer red, hot start polymerase, primers directed against region of HPV high risk group and low risk HPV types. Master Mix was prepared according to kit instructions. 15 ul of prepared master mix and 10 ul of extracted DNA were added to each PCR reaction tube. Then 25 PCR cycles were run using following protocol: Soaking for 15 mins at 950°C followed by denaturation 950°C for 15 mins (1 cycle), annealing at 63°C for 30 seconds (42 cycles), final extension at 72°C for 1cycle for 1 min. Later, gel electrophoresis was done using 1.2% agarose gel. Analysis of results based on the presence or absence of specific bands of amplified DNA in agarose gel was noted.

Statistical analysis

Prevalence of HPV infection is expressed as the number of cases tested. Comparisons of prevalence rates by patients a characteristic was performed using chi-square testing. Factors associated with HPV status were selected on cross-tabulation was analyzed by the use of the chi-square test or Fisher's exact test, wherever appropriate. A logistic regression model was used to determine the effect of multiple factors on HPV status. Results are summarized as ORs and corresponding 95% CIs.

For all statistical analysis, a P value of < 0.05 was considered significant. Statistical analysis was performed using SPSS (SPSS Inc, Chicago, IL version 13) and Epi-Info (version 3) statistical software.

Results

During the one year and five month study (August 2009 to January 2011) 100 patients suffering with oral cancer were included in the study. The characteristics of the population studied showed that there was male preponderance (4:26:1), 69% of the cases were more than 50 years and 31% patients were less than 50 years, 58% belonged to urban area and 42% were from rural area, 35% were tobacco non-chewers and 65% were tobacco chewers. Observation showed that 61% of them were smokers while 39% were non-smokers. Contrary to their tobacco related habits majority of the patients (87%) were non-alcoholic while only 13% were alcoholics. Human papilloma virus was detected in 20% (20/100) of the patients. Distribution of HPV prevalence with respect to demographic data showed that it was more in males 90% (18/20), more in 75% (15/20) of the patients who were more than 50 year, who belonged to urban areas and who were tobacco chewers, 60% were smokers. It was also observed that 75% (15/20) of the patients who had HPV virus were non-alcoholics (Table 1).

The sites of the cancers were divided into oral cancer, oropharyngeal cancer & Head & neck cancer for the convenience of study purpose. Oral cancer occurs in the

| Table: Status of HPV detection, Demography and Addiction in patients with Head and Neck |
|-----------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Characteristic                          | Total (n=100)   | HPV-positive group (n=20) | HPV-negative group (n=80) | Unadjusted OR  |
|                                        | No.  | %    | No.  | %    | No.  | %    | OR(CIs) | P value |
| Sex                                     |      |      |      |      |      |      |         |        |
| Male                                    | 81   | 81   | 18   | 90   | 63   | 79   | 2.42    | 0.263   |
| Female                                  | 19   | 19   | 2    | 10   | 17   | 21   |         |         |
| Age at diagnosis (yrs.)                 |      |      |      |      |      |      |         |        |
| > 50                                    | 69   | 69   | 15   | 75   | 54   | 68   | 1.44    | 0.518   |
| <50                                     | 31   | 31   | 5    | 25   | 26   | 32   |         |         |
| Geographical area                       |      |      |      |      |      |      |         |        |
| Urban                                   | 58   | 58   | 15   | 75   | 43   | 54   | 1.18    | 0.772   |
| Rural                                   | 42   | 42   | 5    | 25   | 17   | 46   |         |         |
| Tobacco exposure(Chewing)               |      |      |      |      |      |      |         |        |
| Chewers                                 | 35   | 35   | 5    | 25   | 30   | 38   | 0.55    | 0.298   |
| Non-chewers                             | 65   | 65   | 15   | 75   | 50   | 62   |         |         |
| Smoking                                 |      |      |      |      |      |      |         |        |
| Non smoker                              | 61   | 61   | 8    | 40   | 53   | 66   | 0.33    | 0.035   |
| smoker                                  | 39   | 39   | 12   | 60   | 27   | 44   |         |         |
| Alcohol intake                          |      |      |      |      |      |      |         |        |
| Non Alcohol drinkers                    | 87   | 87   | 15   | 75   | 72   | 90   | 0.33    | 0.084   |
| Alcohol drinkers                        | 13   | 13   | 5    | 25   | 8    | 10   |         |         |

Then supernatant was discarded without disturbing the pellet. 500ul of washing solution was added to the tubes and centrifuged for 30 seconds at 10,000 g. Again supernatant was removed. Then incubated for 5 mins. at 65°C and vortexed periodically. Pellet was resuspended with 100 ul of DNA elution buffer. Later incubated for 5 min at 65°C and centrifuged for 1 min at 12000 g. And finally supernatant containing the desired DNA was available for amplification. Extracted DNA was stored at -20°C until processed.

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oral cavity which includes lip, tongue & hard palate, and oropharyngeal cancer includes soft palate, base of the tongue, tonsils & places around. The tissue biopsy histopathology showed that almost all were squamous cell carcinomas. Detection of HPV types by multiplex PCR was 20% (20/100). High risk HPV-16 was detected in 35% (7/20) in squamous cell carcinomas of oral and oropharyngeal cancers. Other HPV types detected were 65%. The oral cavity tumors like the buccal mucosa and tongue significantly showed more prevalence of HPV positive as compared to oropharynx (Table 2).

**Discussion**

The incidence of Head and Neck cancers varies widely around the world and also within population. Oral and oropharyngeal cancer contributes 3-5% in Europe while this figure in parts of south East Asia reaches up to 40-50%. Around 80-90% of head and neck cancer cases are associated with risk factors such as smoking, betel nut chewing or tobacco chewing and alcohol abuse. Recent studies have clearly established HPV as a definitive risk factor for oral pharyngeal cancer and it is now a well-defined entity with well-known characteristics that include young age, good performance status, male gender, non-smoking or non-drinking status and high risk sexual behavior. We procured the data for the prevalence rates of head and neck cancers from the community oncology department of our institute and it is 33%, 33.65% and 34.26% in 2009, 2010 and 2011 respectively and there has always been a male preponderance (2.92:1). We included 100 patients suffering with head and neck cancers in our study. The observations showed that there was male preponderance (4.26:1). The study showed 69% of the patients were above the age of 50 years. A report by Jeanne Erdann et al. states that the typical patient belongs to age group of 60-65 years who were old heavy drinkers and smokers. We observed that the conventional risk factors like smoking (61%), tobacco chewing (65%) and age more than 50 yrs were the causative factors for the development of oropharyngeal cancers. In our study HPV was detected in 20% of the cases which is nearer to some studies conducted elsewhere. The study conducted by Dhananjay sarnath et al.,10 showed that the detection of HPV was 34% in patients less than 50 yrs. which was quite high when compared to our study. HPV-positive oropharyngeal cancers comprise a distinct molecular, clinical, and pathologic disease that has a markedly improved prognosis. HPV 16 in our study was prevalent in 45.3% of the cancers. There is a strong association between HPV and oropharyngeal cancers and our findings suggest that HPV-positive oropharyngeal cancer arising from buccal mucosa and tongue have etiological association with high-risk HPV-16. In contrast to HPV-negative oropharyngeal cancers we have seen that they have distinct pathology, risk factors like tobacco chewing, associated with smoking. An etiological link between HPV and non-oropharyngeal tumors is less firmly established. The predominance of oncogenic, high-risk viral types (HPV 16, 18, 31, 33) in HNSCC (16,52) previously identified as the major HPV types in cervical carcinomas worldwide argues for a potentially analogue role for these viruses in development of malignancy in the upper airway. However, similar HPV prevalence estimates have been reported in a population-based study and in case series in the United States and in Europe. The means by which HPV is transmitted to the upper airway is unclear. Although oral HPV infections are rare in newborn children of infected mother and in children prior to sexual activity, infections increase after onset of sexual activity. Epidemiologic studies of cervical cancers have clearly demonstrated that high-risk type mucosa- tropic HPVs are transmitted by sexual contact. Although HPV presence in head and neck cancers has not yet been convincingly linked to the specific sexual practices such as oral sex HPV positivity has been linked to the number of sexual partners in three case-control studies. Therefore this may be another reason for the low identification of HPV types in our group of patients.

**Table 2: Histopathology of the clinically diagnosed Head & Neck Cancers and detection of high risk types of HPV.**

<table>
<thead>
<tr>
<th>HPV types (n=20)</th>
<th>Total</th>
<th>Positive</th>
<th>16**</th>
<th>33**</th>
<th>39</th>
<th>45</th>
<th>52</th>
<th>58</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sr.No</strong></td>
<td><strong>Site of Biopsy</strong></td>
<td><strong>Histopathology</strong></td>
<td><strong>Histopathology</strong></td>
<td><strong>Total</strong></td>
<td><strong>Positive</strong></td>
<td><strong>16</strong></td>
<td><strong>33</strong></td>
<td><strong>39</strong></td>
</tr>
<tr>
<td>1.</td>
<td>Oral cancers</td>
<td>SCC*</td>
<td>73</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-Malignant</td>
<td>11</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2.</td>
<td>Oropharyngeal</td>
<td>SCC</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-Malignant</td>
<td>4</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Head &amp; Neck cancer like mandible, thyroid, maxilla</td>
<td>SCC</td>
<td>7</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-Malignant</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>100</td>
<td>20</td>
<td>7 (35%)</td>
<td>3 (15%)</td>
<td>1 (5%)</td>
<td>2 (10%)</td>
</tr>
</tbody>
</table>

* Squamous cell carcinoma, ** Type 16 & 33 is a high risk HPV.
Conclusion
There has been a shift in the aetiopathogenesis of the head 
and neck tumours. HPV positive oropharyngeal cancer is 
recognized as a distinct subset of head and neck squamous 
cell carcinoma with a good prognostic association in the 
treatment outcome which is independent of age, status, 
tumour differentiation, gender or patient’s habits. It was 
obvious from the study which we undertook that the 
overall detection of HPV-DNA in tumourogenic tissue was 
20% of which HPV-16 accounted for 35%. In rest of the 
patients it can be stated that the other risk factors like age 
more than 50 yrs, old habits of smoking or chewing 
tobacco or the betel nut may have attributed to 
tumourogenesis. More studies on the tumour biology , and 
oncogenes (useful markers) combining with HPV status 
will give a different direction for the precise treatment of 
individual treatment.

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