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Human papillomavirus genotype distribution in oropharynx and oral cavity cancer in France—The EDiTH VI study

Jean Lacau St Guily[a,*], Anne-Carole Jacquard[b], Jean-Luc Prétet[c], Julie Haesebaert[b], Agnès Beby-Defaux[d], Christine Clavel[e], Gérard Agius[d], Philippe Birembaut[e], Claire Okaïs[b], Yann Léocmach[b], Benoît Soubeyrand[b], Pierre Pradat[f,g], Didier Riethmuller[c,h], Christiane Mougin[c], François Denis[i]

[a] *Service d'Oto-rhino-laryngologie et chirurgie cervico-faciale, Hôpital Tenon, Université de Paris 6 et Faculté de Médecine Pierre-et-Marie Curie, Assistance Publique-Hôpitaux de Paris, 4 rue de la Chine, 75970 Paris cedex 20, France*
[b] *Sanofi Pasteur MSD, Lyon, France*
[c] *Laboratoire de Biologie Cellulaire et Moléculaire, CHU J Minjoz, EA3181, Université de Franche-Comté, Besançon, France*
[d] *CNRS UMR 6187, CHU de Poitiers, Laboratoire de Virologie, Université de Poitiers, Poitiers, France*
[e] *INSERM UMRS 903, CHU Reims, Université de Reims, Laboratoire Pol-Bouin, Reims, France*
[f] *Service d'Hépatologie, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Lyon, France*
[g] *INSERM U871, Lyon, France*
[h] *Service de Gynécologie-Obstétrique, CHU Saint Jacques, Besançon, France*
[i] *Laboratoire de Bactériologie et Virologie, CHU Dupuytren, Limoges, France*

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ABSTRACT

Background: The incidence of oropharyngeal cancers has gradually increased over the last decades. Recent studies suggest an association between human papillomavirus (HPV) infection and several head and neck cancers, especially oropharyngeal and oral cavity invasive carcinomas.
Objectives: The objective was to assess the overall and type specific HPV prevalence in oropharyngeal and oral cavity carcinomas in France.
Study design: Paraffin-embedded tumour specimens were retrospectively collected in 12 French centres and centrally tested for HPV detection and genotyping (INNO-LiPA assay).
Results: A total of 523 cases (77% males) were collected, among which 60% were oropharyngeal and 40% oral cavity carcinomas. The most frequent anatomical sites were tonsil (58.9%) and base of tongue (13.7%) for the oropharynx and floor of mouth (41.1%) and oral tongue (38.3%) for the oral cavity. Overall HPV prevalence was 46.5% in oropharyngeal carcinomas and 10.5% in oral cavity carcinomas and was higher in female than in male cases (63.5% vs 42.2% in oropharynx and 17.2% vs 8.0% in oral cavity). About 95% of HPV-positive cases were infected by a single HPV type. HPV 16 was the most prevalent type and was found in 89.7% and 95.5% of HPV-positive oropharyngeal and oral cavity carcinoma cases, respectively. All other HPV types had prevalence below 5%.
Conclusions: Our results indicate that HPV is common among oropharyngeal and oral cavity carcinoma cases in France and emphasize the predominance of HPV 16. The potential benefit of HPV vaccination on the occurrence of head and neck carcinomas should be further evaluated.

1. Background

Head and neck (HN) cancer represents the fifth most common malignancy worldwide.[1] France has the highest incidence in Europe with more than 16,000 new cases in 2005 and 5400 deaths.[2] Medical prognosis is poor with a five-year survival rate below 50%.[3] The majority of HN cancers are squamous cell carcinomas (SCCs). HN carcinomas constitute a heterogeneous group involving different anatomical sites. Despite an overall marginal decline in the incidence of some HN cancers in the recent years, the incidence of oropharyngeal SCC has increased substantially, especially in developed countries.[4–6]

Epidemiological and molecular data have suggested a possible association between human papillomavirus (HPV) and a subset of HNSCC[7–9] especially among subjects without established risk factors such as tobacco and alcohol use.[10] Some authors reported

Abbreviations: HN, head and neck; HPV, human papillomavirus; HR, high-risk; LR, low-risk; SCC, squamous cell carcinoma.

* Corresponding author. Tel.: +33 01 56 01 62 24; fax: +33 01 56 01 70 10.
E-mail address: jean.lacau@tnn.aphp.fr (J. Lacau St Guily).

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that HPV-associated HNSCC arises most commonly in the lingual and palatine tonsils.[11] Published studies from Sweden showed that the increased incidence of base of tongue and tonsillar SCC could be explained by a rise in the proportion of HPV-positive tumours which varied from 23% in 1970s to 57% in 1990s and above 80% after 2000.[12,13]

Although HPV-DNA has been identified in HNSCC, discrepancies in HPV prevalence are reported in the literature[14] which hampers the clarification of a possible relationship between HPV and HN carcinogenesis. These discrepancies could be partly explained not only by the methods used to determine HPV prevalence (specimen type, method of sample storage, HPV detection procedure) but also by the lesion site (oral cavity, oropharynx, larynx).[15]

2. Objectives

French data on HPV prevalence in HN cancers are scarce. The objective of this study was thus to assess the overall and type-specific HPV prevalence in a large collection of oropharyngeal and oral cavity histological samples.

3. Study design

3.1. Histological specimens

This French retrospective multicentre study involved 12 centres located throughout the country. To be included, histological specimens should have been collected from year 2000 to 2009, present a validated diagnosis of oropharyngeal or oral cavity invasive carcinomas and be fixed in 4% buffered formalin before paraffin embedding. Cases with ambiguous diagnosis were excluded.

Oropharyngeal invasive carcinomas were classified into the following anatomical sites: tonsil, base of tongue, vallecula, soft palate and uvula, oropharynx lateral wall, and oropharynx posterior wall whereas oral cavity invasive carcinoma cases were classified as: oral tongue, floor of mouth, gingivae, cheeks, intermaxillary commissure, hard palate, and lips.

3.2. HPV genotyping

DNA isolation and HPV genotyping were centrally performed at the Department of Cellular and Molecular Biology in Besançon as described previously.[16] Genotyping was performed with the INNO-LiPA HPV Genotyping Extra test (Innogenetics, Gent, Belgium) allowing the identification of 28 different HPV genotypes as well as the HLA-DPB1 gene as internal control for DNA quality. As recommended by the manufacturer, only samples positive for any HPV and/or for the HLA-DPB1 gene were included in the analysis.

To be consistent with previous EDiTH studies[17–19] the 28 HPV genotypes identified by the INNO-LiPA assay were classified as follows:

Low-risk (LR) HPV: HPV 6, 11, 26, 40, 43, 44, 53, 54, 66, 70, 71, 73, 74.
High-risk (HR) HPV: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, 82.

In case of multiple HPV infection and if at least one HR genotype was present, the sample was considered as HR. If only LR genotypes were present, the sample was considered as LR.

3.3. Patient data

Data on gender, age at diagnosis, area of residence, medical history of radiotherapy, as well as histological diagnosis and type of sampling (biopsy or surgical specimen) were collected for each case.

3.4. Statistical analysis

Since the objective of this study was to describe the genotype-specific prevalence of HPV in oropharyngeal and oral cavity carcinoma cases, the sample size had to be large enough to allow the description of rare genotypes with high enough precision. Therefore the sample size was calculated using an estimated HPV prevalence of 30% in oropharyngeal invasive carcinomas and 20% in oral cavity invasive carcinomas giving a necessary number of subjects of 530 (303 oropharyngeal carcinomas and 227 oral cavity carcinomas).

All statistical analyses were performed using SPSS v.11.5 for Windows. A p-value below 0.05 was considered as statistically significant.

3.5. Ethical consideration

According to the French legislation (Public Health Code modified by the law no. 2004-806, August 9, 2004 and the Huriet-Sérusclat act 88-1138, December 20, 1988) and since this study only involved data extracted from medical records and stored histological specimens, no informed consent from the patients was necessary. Data collected from participating pathology centres were strictly anonymous.

4. Results

4.1. Samples' characteristics

A total of 616 histological samples of oropharynx or oral cavity invasive carcinomas were retrospectively collected. Ninety-three samples (15.1%) were excluded from the analysis because neither HPV nor cellular DNA could be amplified.

Among the 523 remaining samples, 314 (60%) were oropharyngeal carcinomas and 209 (40%) were oral cavity carcinomas. About 77% of all cases were in males and this percentage differed between oropharyngeal and oral cavity carcinomas (79.9% vs 72.2%, respectively; $p = 0.041$). Age at diagnosis was similar in both carcinoma groups (59 years) and did not vary according to gender. Most tumours (98.5%) were SCC. In eight cases (1.5%), the histopathological diagnosis was not available.

The most frequent carcinoma sites were tonsil (58.9%) and base of tongue (13.7%) for oropharyngeal carcinomas and floor of mouth (41.1%) and oral tongue (38.3%) for oral cavity carcinomas.

4.2. HPV prevalence

Overall HPV prevalence was 46.5% in oropharyngeal invasive carcinomas and 10.5% in oral cavity invasive carcinomas ($p < 0.001$) and was higher in female than in male cases (63.5% vs 42.2% in oropharyngeal carcinomas; $p = 0.002$ and 17.2% vs 8.0% in oral cavity carcinomas; $p = 0.049$) (Table 1). Mean age at diagnosis was similar in HPV positive and HPV negative among oropharyngeal carcinomas (59.8 vs 59.1 years) and among oral cavity carcinoma cases (59.7 vs 59.1 years). Among oropharyngeal carcinoma cases, the HPV prevalence varied according to geographical area of residence being higher in the Paris area (60.9%) and in the Western part of the country (50%) than in the Eastern area (35–9%; $p = 0.022$). Among oral cavity carcinoma cases, the highest prevalence was also observed in the Paris area (18.2%) although the difference between all areas was not significant ($p = 0.53$). The HPV prevalence differed also according to the recruitment centres ranging from 21% ($n = 14$) to 87% ($n = 39$) for oropharyngeal carcinomas and from 0% ($n = 31$) to