abstract

Objective: The aim of this work was to determine the molecular prevalence of the Human Papilloma Virus (HPV) in laryngeal carcinomas and to identify its circulating genotypes.

Patients and methods: This was a descriptive, retrospective study of 20 years which included all the patients followed in otolaryngology department for laryngeal carcinoma. The virological analysis was done on the laryngeal biopsy pieces included in paraffin using the Gene Xpert technique. This technique uses real-time PCR to identify oncogenic HPV genotypes.

Results: A total of 108 patients with laryngeal carcinomas were collected. Among them, 21 samples were associated with oncogenic HPV, a molecular prevalence of 19.4%. These were the HPV 18/45 (14.28%), HPV-16 (28.57%) and the group of HPVs with high oncogenic risk other than 18/45 and 16 (57.15%). All of these patients were male, mean age was 35.71 ± 3.17, and 85.7% of them were under 40 compared to patients with carcinomas not associated with HPV (P=0.0003). Oral-genital contact was the main risk factor for contamination in all of these patients (P=0.0002). The HPV-positive laryngeal carcinomas were all micro-invasive and the patients had better survival compared to those who had HPV-negative carcinomas (P=0.0001).

Conclusion: HPV-positive laryngeal carcinomas are most often observed in subjects under 40 years of age with good survival at 12 months. The circulating genotypes in Brazzaville are 16, 18/45 and the group of oncogenic HPV other than 16 and 18/45.

Keywords: Larynx, Cancers, HPV, Brazzaville.

Introduction

Carcinomas of the larynx have known risk factors, in particular alcohol intoxication and professions making use of the voice [1], but more and more cases of laryngeal carcinomas without identified risk factors are described in the literature [2]. Are there other factors that would be involved in the development of larynx carcinomas? Several studies have advanced the hypothesis of the role of viruses in the carcinogenesis of the larynx [3,4] and some incriminate genotypes 16 and 18 of the Human Papilloma Virus (HPV) [1-3,5]. However in Congo no study to date has verified this hypothesis. Hence the choices of this present work whose objective was to determine the molecular prevalence of HPV in laryngeal carcinomas and to identify the oncogenic genotypes.

Patients and Methods

The department of ENT, head and neck surgery
at the Talangaï reference hospital and that of the Brazzaville university hospital center were part of a 20-year study (from January 01, 2000 to December 31, 2019). This was a descriptive, retrospective study including all the patients followed for larynx carcinoma with histological evidence. The necessary patient information was collected from well-kept medical records, including all epidemiological, clinical and para-clinical data. The virological analysis for HPV was carried out on biopsy pieces of the laryngeal mucosa embedded in paraffin. It was carried out in two stages, namely: the extraction as well as the assay of viral DNA using the extraction kit (ReliaPrepTM gDNA tissue Miniprep System) and the identification of the types of papilloma virus by genotyping in using the Gene Xpert technique with real-time PCR. This technique classifies oncogenic HPVs into three types: 16, 18/45 and high risk oncogenic HPVs other than 16 and 18/45. Thus the parameters studied were epidemiological (frequency, age, sex), clinical (location of the tumor, risk factors and lifestyle) and biological (anatomopathological, virological). For data analysis, the $X^2$ test was used for the comparison and correlation of several observed distributions in order to define the independence of two qualitative variables. The comparison of the quantitative variables was made by the Student test. The significance threshold was set at $p<0.05$.

Results

A total of 108 patients with laryngeal carcinomas were collected, corresponding to 5.4 cases per year. The association with oncogenic HPV was observed on 21 samples, representing an overall molecular prevalence of 19.4%. The prevalence of the genotypes identified on all positive samples were as follows: HPV-18/45 ($n=3$, or 14.28%), HPV-16 ($n=6$, or 28.57%) and the group HPVs with high oncogenic risk other than 18/45 and 16 ($n=12$, or 57.15%). 85.7% of patients with HPV-positive laryngeal carcinomas were under 40 years of age compared to those with laryngeal carcinomas not associated with HPV ($P=0.0003$). In this group of HPV-positive laryngeal carcinoma patients, the mean age was 35.71 ± 3.17 years (range: 35 and 55 years) and all were male. Sexual behavior through oral-genital contact was the main risk factor found in this group ($P = 0.0002$). Pathologically, the presence of koilocytes testifying to viral impregnation was found in all cases of HPV-positive laryngeal carcinomas. All cases of laryngeal carcinoma associated with HPV were micro-invasive compared to carcinomas not associated with oncogenic HPV ($P = 0.0001$). Tables 1 and 2 present the association between HPV infection respectively with the epidemiological-clinical and anatomopathological characteristics. All patients received treatment regardless of HPV serology. In the group of patients with HPV-positive laryngeal carcinomas seven (7) had undergone laryngectomy

| Epidemiological and clinical characteristics | HPV infection | $P$ |
|---------------------------------------------|---------------|-----|
|                                            | HPV + n (%)   | HPV – n (%) |
| Age (years)                                 |               |     |
| $< 40$                                      | 18 (85,7)     | 0   |
| $[40 - 55[$                                | 3(14,3)       | 33 (38) |
| $\geq 55$                                   | 0             | 54 (62) |
| Sex                                         |               |     |
| Men                                         | 21 (100)      | 69 (79,3) |
| Women                                       | 0             | 18 (20,7) |
| Risk factors                                |               |     |
| oral-genital contacts                       | 21 (100)      | 1 (1,1) |
| Alcohol-tabacco                             | 2 (9,5)       | 87 (100) |
| Tumor site                                  |               |     |
| Glottic                                     | 0             | 3 (3,5) |
| Glottic and sus-glottic                     | 21 (100)      | 84 (96,5) |
| Patient survival (months)                   |               |     |
| $< 6$                                       | 21 (100)      | 87 (100) |
| $[6 -12[$                                  | 21 (100)      | 48 (55) |
| $\geq 12$                                   | 21 (100)      | 0 |

$n$: Effective, $\%$: Percentage

Table 1: Association between HPV infection and epidemiological and clinical characteristics.
alcohol-related laryngeal cancers and smoking has fallen by 2.42% each year since 1983 [9].

The Gene Xpert technique with real-time PCR used in this study made it possible to identify genotypes 16, 18/45 and other HPVs with high oncogenic risk. In the latter group, which includes types 31, 33, 35, 51, 52, 56, 58, 59, 68 and 82, the Gene Xpert automaton that we have used makes no difference. It emerges from this virological analysis that it is the HPVs of this group which were the most represented well before types 16 and 18/45. However, this distribution seems to depend on the geographic origin of the patients. This is the case of the studies carried out by KREIMER et al, SI-MOHAMED et al all from North America who report in their respective series that it is rather the HPV-16 genotype which is predominant in laryngeal carcinomas followed by genotype 18 and other high oncogenic risk HPV contrary to our results [10,11].

Laryngeal carcinomas associated with oncogenic HPV were mainly found (85.7%) in men under 40 years of age, unlike the group of carcinomas not associated with HPV which are the prerogative of patients over 40 years of age (P = 0.0003). These patients under the age of 40 all had an oral-genital sexual practice which, according to the literature, represents the main mode of contamination of HPV in the upper aerodigestive tract [12,13]. Several authors have reported a high prevalence of HPV-positive laryngeal carcinomas well above tobacco-induced carcinomas in young subjects [14,15].

If the tumor invasion determines the course of the disease, this will explain the better survival observed in the group of patients with HPV-positive laryngeal carcinomas since it appears from the present study that these carcinomas are micro-invasive. CHERNOCK et al, SHOUSHTARI report in their different series that laryngeal carcinomas caused by HPV are most often micro-invasive and of slow evolution whereas those induced by tobacco are almost always invasive even several years after stopping tobacco consumption [14,15]. As a result, HPV infection seems to be a factor of good prognosis insofar as survival remains better after treatment as was observed in the present study. This favorable clinical development is probably due to better chemosensitivity or radiosensitivity than that of carcinomas not induced by viruses [16,17]. However, HPV-positive smoking patients have a poorer prognosis than non-smoking HPV-positive patients [18]. In all cases, young age remains a factor of good prognosis thanks to the rarity of co-

| Histopathology | HPV infection | | | | |
|---|---|---|---|---|---|
| | HPV + n (%) | HPV – n (%) | P | |
| Types | | | | |
| Squamous cell carcinoma | 21 (100) | 87 (100) | P > 0,05 | |
| Invasion | | | | |
| Invasive carcinoma | 0 | 87 (100) | 0,0001 | |
| Micro-invasive carcinoma | 21 (100) | 0 | | |
| Differentiation | | | | |
| Good | 21 (100) | 87 (100) | P > 0,05 | |
| Way | 0 | 0 | | |
| Little | 0 | 0 | | |

Table 2. Association between HPV infection and histopathological characteristics.

without additional radiotherapy (33.3%) and 14 had undergone palliative chemotherapy (66.7%). At the end of the study, the 12-month survival was 100% in the group of patients with HPV-positive laryngeal carcinomas while it was zero in patients with carcinomas not associated with HPV (P=0, 0001) (Tables 1 and 2).

Discussion

The present study reports 5,4 cases of laryngeal carcinoma each year in Congo, which makes this type of cancer a frequent pathology in otolaryngology as also reported in the literature [6,7]. The overall molecular prevalence of oncogenic HPV in laryngeal carcinoma was around 19.4%. This prevalence of oncogenic HPV would be increased if the virological analysis was done on the laryngectomy surgical parts, while we used the laryngeal biopsy parts. This could explain the large number of negative samples since some biopsies would certainly be done in uninfected areas. So good practice would rather be operative parts of laryngectomy in order to give maximum possibilities on virological analysis in search of oncogenic HPV. The literature reports that HPV-positive laryngeal carcinomas are frequent and their prevalence varies according to the regions of the world. This is the case of DAYYANI et al who report prevalence of 45% and 80% respectively in France and in North America [8]. Other authors like CHATURVEDI AK et al in the United States of America report an increase in the annual incidence of HPV-positive laryngeal cancers by 0.65% while that of alcohol-related laryngeal cancers and smoking have fallen by 2.42% each year since 1983 [9].
morbidity. This difference was observed in the present study because all patients with HPV-positive laryngeal carcinomas were less than 40 years old and had a significantly better 12-month survival than that observed in patients with non-associated laryngeal carcinomas to HPV.

Conclusion

HPV-positive laryngeal carcinomas are most often observed in men under 40, the circulating genotypes in Brazzaville are 16, 18/45 and the group of oncogenic HPVs other than 16 and 18/45. Oral-genital contact is the main risk factor and survival at 12 months is 100% after treatment.

Conflict of Interest

The authors declare no conflict of interest in relation to this article.

Contribution of Authors

- Otouana Dzon HB, Ngouoni GC, Diembi S: design, documentary excavation, writing;
- Ondzotto GW, Tsierie-Tsoba A: writing of the discussion;
- Itiere Odzili FA, Ondzotto G: critical reading.

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