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Study of Epidemiology and Human Papilloma Virus Prevalence in Oral Cavity Cancers

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Abstract

Oral cavity cancers (OCC) are the most common malignancies in the subcategory of head and neck cancers, and represent the 6th most common cancer in the world. These cancers have become more frequent in individuals without a history of alcohol-tobacco abuse, which are the major risk factors. Other factors have been suggested, such as viral infections, but especially genetic alterations. This work establishes the epidemiological profile and researches the presence of viral DNA in OCCs. The epidemiology was highlighted with 105 patients using the Epi Info software. HPV DNA was sought in 50 samples of diseased tissue and blood by attempting to amplify its L1 region by PCR. At the epidemiological level, the results show a mean age of 53.2 years, a sex ratio of 0.8 and a low consumption of tobacco (16.2%) and alcohol (4.8%). HPV was not detected in any of the samples. Thus, the epidemiological profile of OCCs in Senegal is different from that in other countries, and HPV is not associated with its occurrence.

Keywords: *Cancer, Oral cavity, HPV, Epidemiology*

Introduction

The epidemiology of cancers of the oral cavity is part of the more general framework of cancers of the upper aerodigestive tract (UADT) [1]. They account for approximately 25 to 30% of UADT cancers [2]. In general, they appear from the sixth decade of life [3] with alcohol and tobacco being identified as the main risk factors. They are ranked as the 6th most common cancer worldwide, and 3rd in developing countries [4]. Globally, they had 354,864 new cases in 2018, or 2% of all cancers, and approximately 177,384 cases of deaths, or 1.9% of cancer deaths [5]. Men account for 69.4% of cases, with a higher cumulative risk of dying before age 75. Age-standardized incidence rates are lower in West Africa, with little difference between men and women at 1.2 and 1.1 cases per 100,000 persons/year, respectively [5,6]. The five most affected countries are India (77,003 cases), the United States (26,064 cases), China (21,413 cases), Pakistan (12,761 cases), and Bangladesh (10,550 cases) [7]. Reports have shown that the global incidence is higher in more developed regions, but mortality is higher in less developed regions, which reflects social inequality [8].

According to GLOBOCAN [9], it is the 16th most common cancer in Senegal, with 130 new cases during the last 5 years and 111 deaths. The average age is about 52 years and the female sex predominates, with the majority being non-alcoholic-tobacco users [10,11]. In Africa in general and in Senegal in particular, data on cancers of the oral cavity are scarce and besides that the epidemiology differs from other countries.

Based on epidemiologic and clinicopathologic evidence, it has been proposed that Human Papillomavirus (HPV) infection is linked to the development of oral cancer [12]. HPV is one of the most common sexually transmitted infections and belongs to a large family of viruses, the papovaviridae. They are small (about 55 nm in diameter) and epitheliotropic. Their genome is composed of 7,200 to 8,000 base pairs with molecular weights of 5.2 x 10^6 daltons. They have a double-stranded circular DNA with a capsule of 72 capsomers of icosahedral structures, without a lipoprotein envelope [13]. Numerous papillomaviruses are known, with over 150 types; however, not all genotypes are considered carcinogenic [14]. Based on their potential oncogenic activity, HPV subtypes have been divided into high-risk (HPV-HR) and low-risk (HPV-LR) viruses. HPV-HR are associated with cancer development and are called viral "oncogenes" [15]. The prevalence of HPV in normal oral mucosa (latent infection) and its relationship to oral cancer have generated conflicting opinions. Most of the published studies have included several head and neck subsites, which have prevented specific analysis of HPV involvement in oral carcinogenesis [16]. In addition, the frequency of HPV infection in oral cavity cancer shows a lot of variation between studies around the world [17]. To support the implication of HPV in oral tumors, few studies have been conducted to determine the frequency of HPV DNA exclusively in squamous cell carcinoma of the oral cavity, particularly in Senegal. Hence, this study aims to update the epidemiological profile of oral cavity cancers and to detect the presence of HPV in them.

Methodology

This study was approved by the Research Ethics Committee of Cheikh Anta Diop University (Reference: Protocol 0272/2018/CER/UCAD). One hundred and five (105) patients diagnosed with OCCs between March 2017 and October 2020, at the Department of Stomatology and Maxillofacial Surgery of the Hospital Center University Aristide Le Dantec in Dakar were the subject of this study. Demographic, clinico-pathological, and etiological data were collected from the patients' clinical records and then entered with Microsoft Excel 2016 spreadsheet for statistical analyzes, thereby allowing the description of the epidemiological profile of OCC in Senegal. Epi Info software version 7.2.4.0 enabled these analyses to be carried out by providing, among other things, the number of patients, the frequency, and the 95% confidence interval for each parameter studied. For statistical tests, a p value <0.05 is considered significant.

DNA extraction was performed from blood and tissue using the Zymo research kit and the Purelink viral RNA/DNA kit according to the manufacturer's conditions. In order to test for the presence of viral DNA in the OCCs, the L1 gene was amplified using the primer pair (MY09/11). PCR was performed using 25 µl of master mix, 1 µl of forward primer, 1 µl of reverse primer, 1 µl of $MgCl_2$, 20 µl of ultrapure water and 2 µl of DNA. The following conditions were used: 94°C for 5 min; 35 cycles (94°C for 30 s, 55°C for 30 s, 72°C for 1 min); 72°C for 15 min. A positive control (PC) for cervical cancer was used.

Results

Characteristics of the Population

The clinical parameters of the one hundred and five (105) patients enrolled in this study are listed in Table 1. More than half (55.4%) come from the different regions of Senegal. Age at diagnosis ranged from 22 to 90 years, with 38.1% of patients aged between 50 and 64 years old. There was a slight predominance of women, with a sex ratio of 0.8, and they were older than men (55.7 years *vs.* 49.9 years) with a non-significant p-value of 0.12. Histologically, 93.3% of cases are squamous cell carcinomas, and are generally well differentiated (64.8% of cases). Different structures of the mouth are affected: the gum (30.5% of cases), the tongue (17.1%) and the inner face of the cheek (15.2%) are the most affected. Among the patients whose tumor size was reported, the majority (23 cases) were larger than 4 cm in size. The presence of lymphadenopathy (s) was noted in 21.9% of patients, and 48.6% were at an advanced stage (stage III or IV). The rate of alcohol and tobacco use was low, with only 16.2% of smokers and 4.8% of alcohol users. Note that 21.9% of cases have poor oral hygiene.

Table 2 shows the distribution of age groups in relation to gender. For all age groups, the incidence is higher in women except those under 35 years of age.

Amplification Reactions of the HPV L1 Region

The result of this PCR for 10 cancerous tissue and one positif control is shown in figure 1. The latter shows the absence of viral DNA for our samples, except for the positive control which shows a band, of about 450 bp. The result is the same for all samples (tissue and blood).

Discussion

One of the first activities of this study was to collect tumor samples as well as clinical data from patients with OCC at the Department of Stomatology and Maxillofacial Surgery of the Hospital Center University Aristide Le Dantec. Of the 105 cases, 59 (56.2%) were women as opposed to 46 (43.8%) men. This shows that in Senegal, there is a slight predominance of women in the incidence of OCC, with a sex ratio of 0.8. This sex ratio is identical to that found in Senegal by Dieng *et al.* [11], and not far from the result of Millogo *et al.* [18] in Burkina with a sex ratio of 0.85. In this study, the hypothesis is supported according to which the aesthetic concern would lead women of our societies to consult more often than men as soon as a significant anomaly is noticed in the oro-maxillo-facial sphere. However, oral cancer is considered worldwide as a male pathology and especially in the most affected countries like India [7]. Indeed, in India, a study by Singh *et al.* [19] identified 84.8% of men as opposed to 15.2% of women.

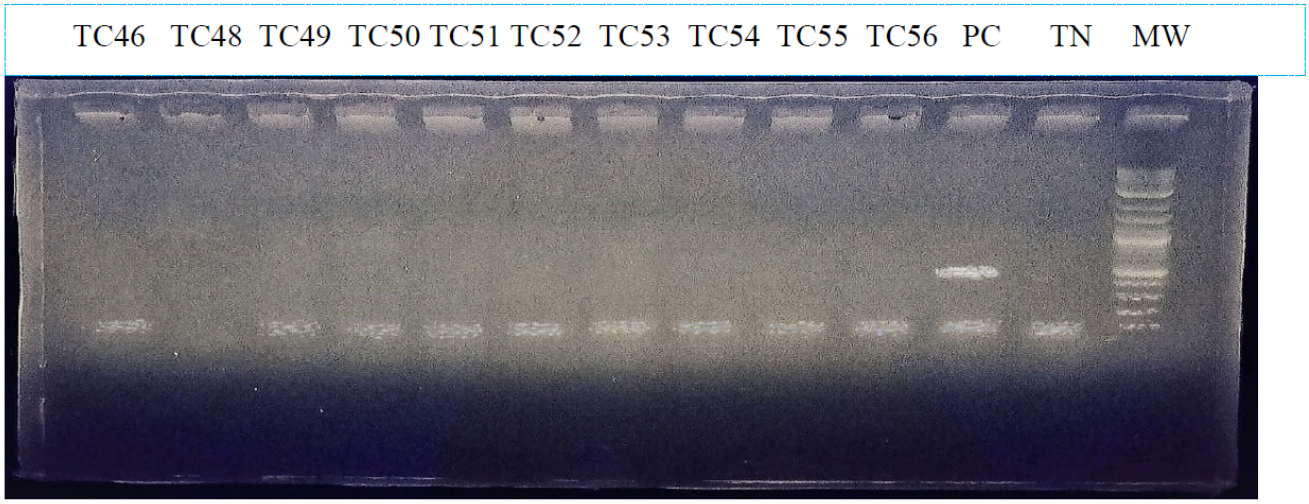

Figure 1: Electrophoretic migration profile of PCR products from the L1 region.
MW: molecular weight; TC = Cancerous tissue PC: positive control; TN: negative control.

Table 1: Epidemiological and clinical characteristics of patients.

Characteristics	Minimum	Maximum	Average
Age (year)			
Overall age	22	90	53.2
Men	22	78	49.9
Women	25	90	55.7
	Number	Frequency (%)	CI (95%)
Gender			
Male	46	43.8	34.1-53.8
Female	59	56.2	46.2-65.9
Sex ratio	0.8		
Age groups			
Under 35 years	19	18.1	11.3-26.8
35 years - 49 years	19	18.1	11.3-26.8
50 years - 64 years	40	38.1	28.8-48.1
65 years and older	27	25.7	17.7-35.2
Origin			
Dakar	41	39	29.7-49.1
Other regions	55	52.4	42.4-62.2
Neighboring countries	9	8.6	4-15.6
Histopathology			
Squamous cell carcinoma	98	93.3	86.7-97.3
Adenoid carcinoma	1	1	0-5.2
Verrucous carcinoma	1	1	0-5.2
Sarcoma	4	3.8	1-9.5
Lymphoma	1	1	0-5.2
Differentiation			
Good	68	64.8	54.8-73.8
Average	15	14.3	8.2-22.5
Weak	8	7.6	3.3-14.5
NA	14	13.3	7.5-21.4
Tumor site			
Gum	32	30.5	21.9-40.2
Tongue	18	17.1	10.5-25.7
Cheek	16	15.2	9-23.6
Lip	8	7.6	3.3-14.5
Palate	6	5.7	2.1-12
Floor	2	1.9	0.2-6.7
Facial mass	9	8.6	4-15.6
Mixed	14	13.3	7.5-21.4
Tumor size (cm)			
$T \leq 2$	3	2.9	0.6-8.1
$2 < T \leq 4$	18	17.1	10.5-25.7
$T > 4$	23	21.9	14.4-31
Large extension	18	17.1	10.5-25.7
NA	43	41	31.5-51
Lymphadenopathy			
Positive	23	21.9	14.4-31
Negative	82	78.1	69-85.6
Stage			
Early (stage I + stage II)	13	12.4	6.8-20.2
Advanced (stage III + stage IV)	51	48.6	38.7-58.5
NA	41	39	29.7-49.1
Common risk factors			
Tobacco	17	16.2	9.7-24.7
Alcohol	5	4.8	1.6-10.8
No Alcohol-smoking	66	62.8	52.9-72.1
NA	17	16.2	9.7-24.7
Oral hygiene			
Poor	23	21.9	14.4-31
Good/NA	82	78.1	69-85.6
TOTAL	105	100	

Table 2: Distribution of the different age groups in relation to gender.

	Men		Women		
Age groups	**Number**	**Frequency (%)**	**Number**	**Frequency (%)**	**Total**
Under 35 years	13	68.4	6	31.6	19
35-49 years	8	42.1	11	57.9	19
50-64 years	14	35	26	65	40
65 years and older	11	40.8	16	59.2	27

P-value=0.11

Cancer is a disease whose risk increases with age. In Europe and America, the average age of patients with OCC is estimated to be around 60 years old [3]. In Africa, the average age range is from 47.8 years in Côte d'Ivoire to 49.15 years in Burkina [18]. In our study, the modal class 50 - 64 years represents 38.1% of the cases (40 patients), with a mean age of 53.2 years for the entire study population. This result is close to those found by Touré *et al.* [10] and Dieng *et al.* [11] with 52.6 and 52.9 years of mean age, respectively. This age difference with developed countries could be explained by the difference in standard of living and therefore easier access to medical care, disfavoring for example poor oral hygiene for these populations [2,18]. Indeed, poor oral hygiene is thought to play a direct role in the occurrence of OCCs [20] and it may play a non-negligible role in Senegal [21]. It was often poor in the study by Touré *et al.* [10], and for the study of Millogo *et al.* [18], all patients experienced poor oral hygiene. In our case, these represent 21.9% of the study population, but with missing data.

Despite the fact that older people are more exposed, our results show that young people are also not spared from the disease, especially among men. In fact, in our results, 38 patients were not yet in their fifties and 19 of them were under 35 years of age, 68.42% of whom were men. Touré *et al.* [10] had observed in their cohort that 38% of patients were under 50 years of age. In many countries of the world, there has been an alarming increase in the incidence of oral cancer, especially among young men [3]. This could be explained by earlier exposure to common risk factors such as tobacco use [7] as is the case in India or Pakistan. In these regions, the average age of patients is between 41 and 50 years and one-third of the population aged 15 years uses tobacco in any form [22]. However, the consumption of these substances (tobacco and alcohol) is not common among patients with OCC in Senegal [10,11]. Our results confirm this with only 17 smokers (16.2% of cases), and 5 alcoholics (4.8%). The low alcohol consumption is explained by the fact that 95% of the population is Muslim [21].

The fact that 93.3% of the cases in our study were squamous cell carcinomas is not surprising, since it is well known that they account for more than 90% of all oral cancers [23]. Most studies have confirmed the predominance of squamous cell carcinomas, but with different frequencies. They were the predominant histological type for: Singh *et al.* [19] for all cases (100%), Dieng *et al.* [11] with 98% of cases, and 55.9% for Millogo *et al.* [18]. The latter support the hypothesis that the predominance of squamous epithelial tissue in the mucosa is the cause of this high frequency of squamous cell carcinomas.

The tongue is one of the most common sites in OCCs with 40% of cases [8] especially in Western countries due to excessive smoking and alcohol consumption [22]. In 2005, according to Touré *et al.* [10], the mandible (24.8%), tongue (21.9%) and maxilla (15.2%) represented the majority sites. The gum (30.5% of cases), tongue (17.1%) and inner face of the cheek (15.2%) are the most affected sites in our study. The fact that the maxillary and mandibular gingiva are grouped together may have caused this high rate for the gingiva. This distribution of tumor sites could be explained by poor dental hygiene: either non-healing after dental extraction, creating an open wound in the gum area; or decayed teeth, traumatizing the cheek or tongue, especially for the latter.

More than half of OCCs were diagnosed at stage III or IV [20]. This was the case in Senegal based on previous studies with an average of 86% of cases diagnosed at advanced stages [10,11]. For 48.6% of the cases in our study, the disease was at an advanced stage (stage III or IV), with the presence of lymphadenopathy in 23 cases (21.9%). However, a lot of data are missing to make an estimate of the stage of the disease in our study population. The diagnosis at an advanced stage shows an irregularity or a late consultation of our population at the level of oral care structures, which can be explained by a weakness at the financial level or by the ignorance for example of the early signs of the disease. Other authors such as Millogo *et al.* [18] point to the omnipresence of traditional medicine as perhaps the first resort in our societies

This epidemiological study has certain limitations, such as the large number of unspecified data for a few parameters, or the failure to take into account other parameters such as occupation. The existence of a register or database of oral cancers, which compiles data from all hospital services receiving patients with this pathology, would allow us to know a little more about this disease and its incidence in Senegal.

Searching for HPV DNA was also one of the objectives of the study. It was done by gene amplification of its L1 region on 50 extracts of cancerous tissue and 50 extracts of blood, and none revealed the presence of this virus. This suggests that there is no significant association between OCCs and HPV infection. Ndiaye *et al.* [21] found only 3.4% HPV-positive cases in a study conducted in Senegal on head and neck cancers (HNC). This study, in addition to our own, shows that the prevalence of HPV in HNCs in Senegal is low. This is more or less the same observation that has been made in some African countries. For example, HPV was found in 6.3% of HNC cases in a study in South Africa; [24] 0.74% in Central Africa for Kofi *et al.* [25]; and like our case, it could not be detected in studies in Mozambique [26] and Nigeria [27]. These results are different from what has been reported in other parts of the world. In a systematic review by Kreimer *et al.* [28] compiling data from 60 studies, the overall HPV positivity was 25.9%; and North American countries were more representative

than Europe or Asia. Ndiaye *et al.* [29] reported a positivity of 31.5% when compiling 148 studies. Ndiaye *et al.* [21] argue that there are ethnic disparities regarding the prevalence of the virus in these cancers, with less of it being found to affect the black race. Indeed, studies in the US have shown this to be the case [30,31]. For example Settle *et al.* [30] found 34% positivity in whites as opposed to 4% in blacks. This racial difference would be explained by risky sexual practices, especially oral sex, which is believed to be more prevalent among whites; but also by genetic differences between the two groups, impacting host immunity or viral integration [27]. It also appears that smoking, besides being an independent risk factor in developed countries, makes infections more likely to persist, thus increasing the risk of developing HPV-related diseases [32].

Furthermore, it is recognized that the highest prevalences of HPV infection in HNCs are found in the oropharynx (45.8%) including the tonsils (53.9%), compared to 24.2% for the oral cavity [29]. Thus, the oral cavity is not the preferred site for HPV in HNCs.

Conclusion

As a result of the heterogeneous etiology and lack of definite prognosis of oral cavity cancers, this study aimed to contribute to a better understanding of the epidemiological and molecular profile of patients with OCC in Senegal. An epidemiological profile different from that of Western or Asian countries was found. Indeed, it is that of a relatively young individual, often of a female gender, nonalcoholic-smoker. Added to this is the absence of HPV in patients with OCC in Senegal. Thus, the risk factors are not yet clearly identified, and this opens the way to the search for other factors such as those related to the environment, lifestyle or diet, but especially genetic events.

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