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Squamous cell carcinoma of the oral cavity: clinical and pathological aspects

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Abstract

Oral cavity cancer is a public health problem as the sixth leading cause of cancer worldwide. Most tumor lesions are detected in stage III and IV, leading to a poor prognosis, five-year survival rate ranging between 10% and 40%. Oral cancer etiology is multifactorial, known still incomplete. The main etiopathogenic factors are exposure to cigarette smoke and alcohol consumption. We conducted a retrospective study of oral cavity tumors hospitalized in 2008–2012 in Oral and Maxillofacial Surgery Clinic of the Emergency County Hospital of Craiova, Romania. Of 143 tumors of the oral cavity, 125 were malignant, and of these, 115 (92%) were represented by squamous cell carcinoma. Tumor lesions were more common in males (69%), patients from rural areas (64%) and those over 50-year-old (87.71%).

Keywords: squamous carcinoma, oral cavity, proliferation factors, genetic mutations.

Introduction

Oral cavity cancer is one of the most common neoplastic lesions of the head and neck. Relatively recent data shows that the incidence of neoplastic lesions of the head and neck is quite high, squamous cell carcinoma being ranked as the sixth worldwide [1, 2]. Only in the United States are diagnosed over 21 500 oral carcinomas each year and more than 6000 Americans die each year because of it [3]. The incidence of head and neck carcinomas varies greatly between different regions of the world [4]. Thus, in North America and the European Union, head and neck cancer represents 3–4% of all cases of cancer diagnosed each year; in contrast, in South Asia and Africa, head and neck cancer has a higher incidence, almost double in regard to the U.S. and the European Union, representing about 8% up to 10% of all forms of cancer. The highest rates of oral neoplasia, as regarding the incidence and mortality in Europe, are registered in Central Europe and France [5, 6]. In the last two decades, the overall incidence of squamous cell oral and pharyngeal carcinoma (SCC) fell in the United States. This downward trend was attributed to the reduction of alcohol and smoking [7, 8]. According to some authors [9, 10] in developing countries where health resources are reduced and oral care is poor, oral cancer is a major health problem, most neoplastic lesions being detected in advanced stages. The fact is that, in general, despite advances in the treatment of many other malignancies prognosis for patients with oral squamous cell carcinoma remains poor.

In this study, we have proposed an evaluation of the clinical and pathological aspects of oral squamous cell carcinomas diagnosed over a five years period in the Oral and Maxillofacial Surgery Clinic of the Emergency County Hospital of Craiova, Romania.

Materials and Methods

We performed a retrospective clinical study and quantified all oral cavity tumoral lesions admitted into the Oral and Maxillofacial Surgery Clinic of the Emergency County Hospital of Craiova, between 2008 and 2012. The study included a total of 143 patients, aged between one and 82 years, both from rural and urban area. To highlight the risk factors we investigated in each patient the following clinical aspects: patients' age, social background, alcohol consumption, smoking, dental hygiene, medical history, tumor location, histo-pathological diagnosis. All data obtained were recorded in the Excel software (Microsoft Romania, Bucharest) and processed using the Statistical Package. We used descriptive and analytical statistical tests to assess distribution of cases and relevant correlations.

Histopathological study was performed in all cases of oral cavity tumors in the Anatomic Pathology Laboratory of the Emergency County Hospital of Craiova. Biological material, fragments of resected tumors respectively, were immediately fixed in 10% neutral formalin solution for 48–72 hours at laboratory temperature (21–23^{0}C) and processed by paraffin inclusion. After inclusion in paraffin there were performed 4-µm thick histological

sections using a rotary microtome (Microm HM350) equipped with a water transfer system of histological sections (STS, Microm). For histological study, we used two stains: Hematoxylin–Eosin and Goldner–Szekely technique green light trichromic.

For immunohistochemical study, out of the paraffin-included material we selected a total of 43 cases of squamous cell oral carcinoma. Out of this biological material, 3-µm thick histological sections were realized and were collected on poly-L-Lysine coated slides, and kept afterwards in the thermostat at 37^0C for 24 hours to increase the adhesion of biological material. Following dewaxing and hydration of histological sections, biological material was incubated for 30 minutes in a solution of 1% oxygenated water (hydrogen peroxide). The sections were then washed in tap water before being boiled in pH 6 citrate solution for 20 minutes to unmask the antigen. After boiling were allowed to cool for 15 minutes, then washed in bisaline phosphate buffer solution (PBS), followed by endogenous peroxidase blocking step in 2% skim milk for 30 minutes. Then, the sections were incubated with primary antibodies overnight at 4^0C, and the next day, the signal was amplified for 30 minutes using the secondary antibody with polymer-based peroxidase, EnVision detection system (Dako). The signal was detected by 3,3'-Diaminobenzidine (DAB) (Dako), and after contrasting with Hematoxylin slides were covered with DPX (Fluka).

For the immunohistochemical study, we used the antibodies described in Table 1.

Table 1 – ***Antibodies used in the immunohistochemical study***

Name	Manufacturer	Clonality	Clone	Specificity	Optimum dilution	Antigenic recovery
P53	Dako	IgG2b k	DO-7	P53 proto-oncogene.	1:50	EDTA, pH 8
Ki-67	Dako	IgG1k	MIB-1	Cellular proliferation marker, highlights only dividing cells.	1:100	Boiling CB, pH 6

Results

Analysis of data recorded in observation charts of patients included in the study allowed us to note that out of the total of 143 tumor lesions, 18 were benign (squamous epithelium with dysplastic lesions, fibro-conjunctive structures, epulis, warts, hemangiomas, angiomyolipomas) and 125 were malignant tumors. Of the malignant tumors, by far, the main histological studied type was the squamous cell carcinoma in 115 (92%) patients. Of these, the type of well-differentiated squamous cell carcinoma was represented by 61 (53.05%) cases, moderate squamous carcinomas were found in 36 (31.3%) cases and poorly differentiated squamous cell carcinoma in 18 (15.65%) cases.

Other types of tumors found in the oral cavity of the studied patients were: three (2.1%) basal cell carcinomas, one adenoid carcinoma, one "*in situ*" carcinoma, six (4.2%) hemangiomas, two osteosarcoma, papillomas and epulis cases and one case each of cyst adenolymphoma, angiomyolipoma and adenocarcinoma.

As regards the staging of studied squamous cell carcinomas, most of the cases, 85 (73.91%) patients respectively, were diagnosed with stage III and IV tumors, 28 (22.60%) patients in stage II and only two (1.73%) patients with "*in situ*" squamous cell carcinoma.

Location of tumor lesions was extremely varied. Most of the tumors were located on the lower lip in 52 (36.4%) patients, on the mandible in 36 (25.2%) patients, followed by the mouth floor in 18 (12.6%) patients and the tongue in 12 (8.4%) patients. Other locations included the oral commissure and upper lip (eight cases each, 5.6% each respectively), base of the tongue and upper jaw (four cases each, 2.8% each respectively); the fewer tumors were observed in the spring chin (three cases, 2%) (Figures 1–3).

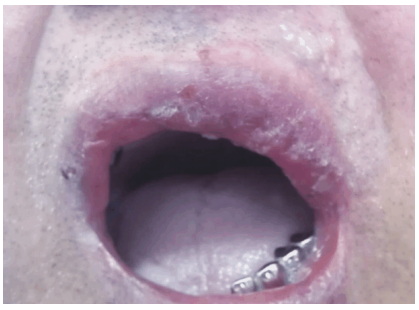

Figure 1 – ***Squamous cell carcinoma of the upper lip.***

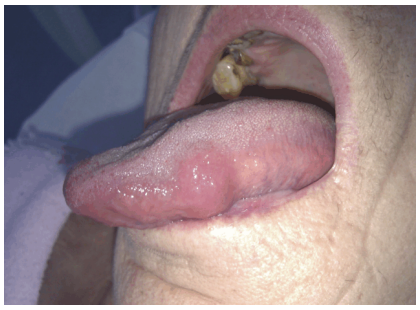

Figure 2 – ***Tongue infiltrating tumor (carcinoma).***

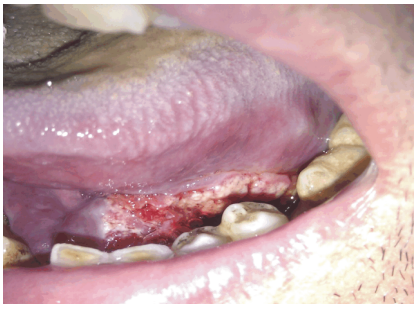

Figure 3 – ***Floor of the mouth tumor.***

Evaluating correlation with social environment, we found that the area of origin of patients with tumor lesions was predominantly rural, this being registered for 91 patients, representing 64%, compared to urban where there were recorded a number of 52 patients, representing only 36% of all patients in the study. In other words, about two thirds of patients with oral cavity tumor lesions were from rural areas and only 1/3 of the urban environment.

In our study, tumor lesions of the oral cavity occurred mainly in male patients. Thus, out of the 143 patients with tumoral lesions of the oral cavity, 96 (69%) were males and 45 (31%) patients were females. Our data confirm other studies that have shown that males consume more alcohol and are chronic smokers compared to female persons. Sex ratio (2/1) was clearly against the male sex.

Compared to the entire group, only 39 (27.2%) patients said they had various viciousness: 13 patients

were smokers, four were alcohol consumers and 22 patients said they were smokers and alcohol consumers.

Patient ages were between one and 87 years, with a mean of 63±14.45 years. Following distribution by age, we observed only three cases between 1–10 years, one case of 13-year-old, 21–30 years decade having no representative. Most of the patients were 50-year-old, with a peak in the decade 61–70 years (Figure 4).

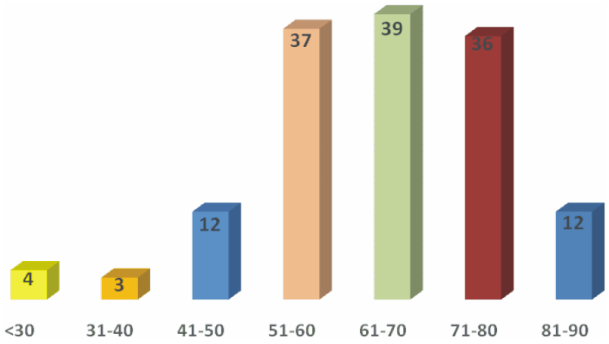

Figure 4 – ***Oral cavity tumors distribution by age.***

Analysis of the group with regard to dental hygiene has shown its absence in a total of 76 (53%) patients, compared to a satisfactory dental hygiene present in 67 (47%) patients (Figure 5).

Most patients had dental tartar, plaque, root debris with periodontitis phenomena. The presence of dental tartar and dental plaque is a local irritation factor and maintain an inflammatory condition of the oral mucosa that predispose to dysplastic or neoplastic lesions.

Histopathological exam

Well-differentiated squamous carcinomas were composed of well-differentiated squamous cells, arranged as islands of various shapes and sizes, with keratin pearls inside, resulted in a process of "neoplastic maturation" (Figure 5).

Inside the keratosic pearls cells appeared acidophil, with pyknotic nuclei, with karyolysis, while the rest of cells had nuclei of various shapes and sizes, much larger than normal epithelium nuclei, with conspicuous nucleoli and heterogeneous chromatin, sometimes disposed as coarse. Most cells showed polyhedral aspect with visible intercellular spines.

Moderately differentiated carcinomas were composed of cords or islands of neoplastic atypical epithelial cells, oval-shaped, oblong, round which infiltrated the tumoral stroma. At the periphery, carcinoma islands were separated by fibrous stromal elements or inflammatory type cells (Figure 6). Nuclei of neoplastic cells had various shapes and sizes, most of them being hypochromic with large nucleoli. Tumor cells often appeared as atypical cells diffusely scattered in the stroma of oral, lingual or labial mucosa, with rare intercellular bridges. Often tumor cells had large, deformed nuclei, hyperchrome or hypochromic, with pinholes and buddings, with multiple atypical mitosis.

Poorly differentiated squamous cell carcinomas appeared as cellular cords, islands or epithelioid-like cells of various shapes and sizes, without resemblance to the epithelium of which they arose (Figure 7). These different aspects of cancer cells make us believe that malignant tumors are heterogeneous entities, multicellular, containing multiple cell lines whose interactions with each other and with the extracellular matrix through paracrine secreted soluble molecules are dynamic and promote cell proliferation, movement and differentiation of neoplasia.

Immunohistochemical study

P53 protein evaluation

The p53 protein is one of the most used immune markers in the study of tumoral processes, mainly for evaluating tumors prognosis by immunohistochemical evaluation of p53 gene mutations. P53 tumoral suppressor gene plays a central role in controlling cell cycle progression from G1 phase to S phase. Changes of this gene allow rapid multiplication of cancer cells, leading to uncontrolled proliferation and increased immunohistochemical expression of p53 protein. In our study, the response to p53 was intensely positive in 37 cases of squamous cell carcinomas, so in about 86% of histological specimens investigated (Figure 8).

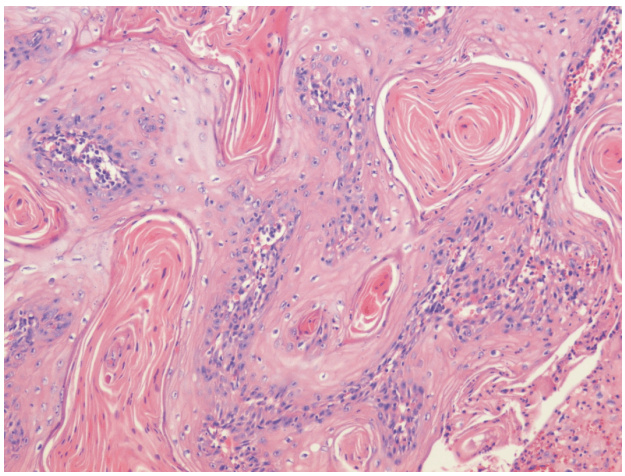

Figure 5 – ***Well-differentiated squamous cell carcinoma of the lingual mucosa. HE staining, ×200.***

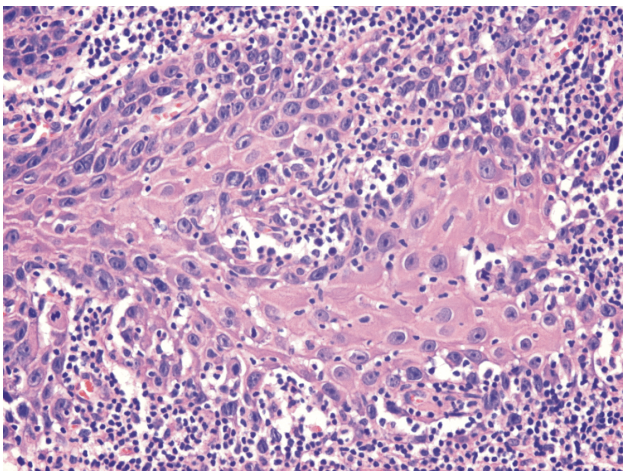

Figure 6 – ***Moderately differentiated, invading lingual carcinoma, with cells arranged in cords. Strong chronic inflammatory infiltrate in the peritumoral stroma. HE staining, ×200.***

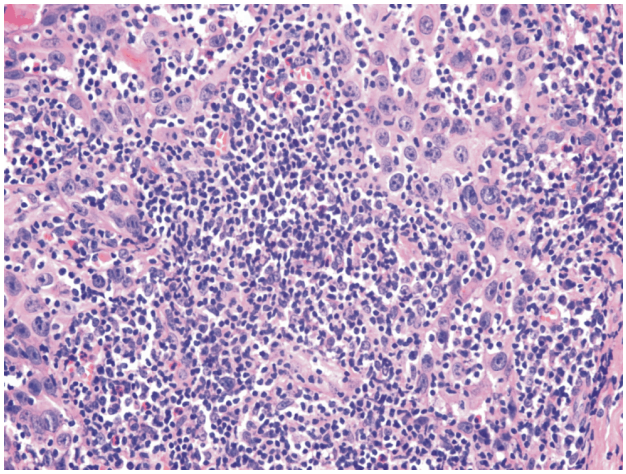

Figure 7 – ***Poorly differentiated carcinoma of the mouth floor with cellular pleomorphism. HE staining, ×200.***

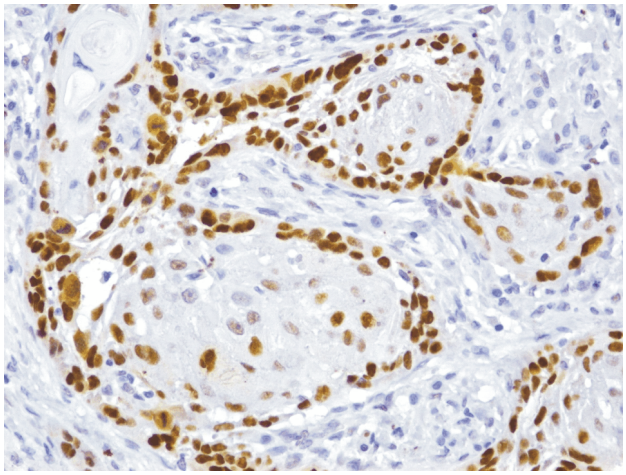

Figure 8 – ***Squamous cell carcinoma of the tongue, well-differentiated, with intense positive reaction of the proliferating cells. P53 immunostaining, ×200.***

Ki-67 proliferating factor evaluation

Ki-67 is a nuclear protein whose expression is strictly associated with cell proliferation and which is widely used in pathology as "proliferation marker" in order to assess the cellular growth fraction in human tumors. Being a factor which gives data on cell proliferation, Ki-67 is frequently used in positive and differential diagnosis of neoplasia, dysplastic lesions, in the study of various tumors. An increase of the number of Ki-67 positive nuclei indicates an acceleration of mitotic rate. In our study, the Ki-67 immunohistochemical reaction occurred more intensely positive in poorly differentiated carcinomas compared with well-differentiated carcinomas (Figures 9 and 10).

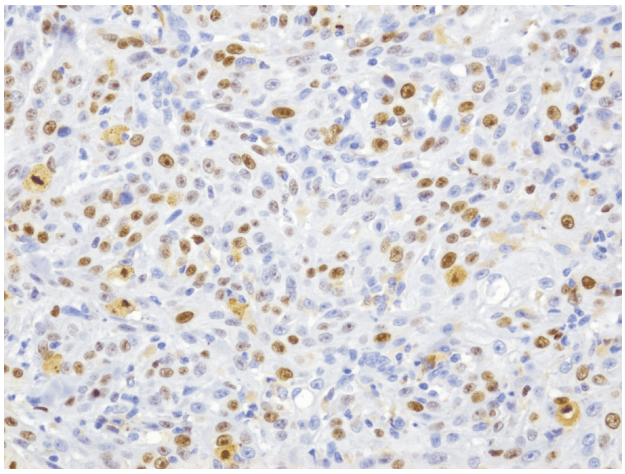

Figure 9 – ***Moderately differentiated squamous cell carcinoma with intense positive reaction to Ki-67. Ki-67 immunostaining, ×200.***

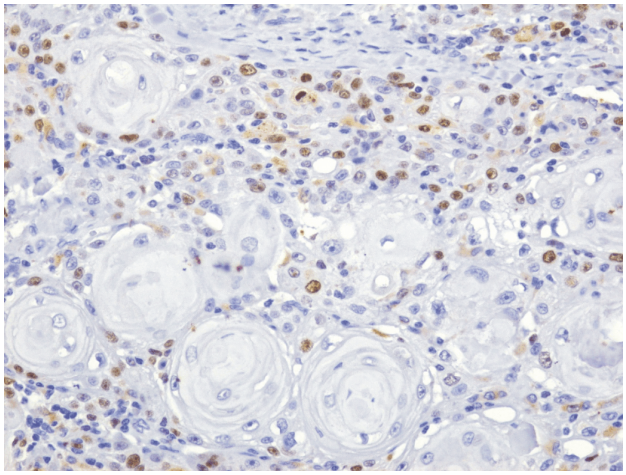

Figure 10 – ***Well-differentiated squamous cell carcinoma with moderate positive reaction to Ki-67. Ki-67 immunostaining, ×200.***

Discussion

Oral cavity cancer is one of the most common forms of cancer with significantly reduced five-year survival rate. Although the oral cavity can be easily examined and the existence of premalignant or malignant lesions could be detected in early stages, most cases of oral cancers in our study were detected late. Thus, about 73.91% of the study group patients were diagnosed with stage III and IV tumors. According to some studies, approximately 60% of cancers are belatedly diagnosed, in stages III or IV, with survival rates ranging from 10% to 40% after five years [11, 12]. Belated detection of tumor lesions in the oral cavity may be because symptoms are extremely low, and patients neglect first symptoms of lack of health education. It should also be noted that national screening programs do not exist, making early detection of malignant and premalignant lesions of the oral cavity to be coincidental, during dental exams. For patients undergoing regular medical checks at the dentist, detection of premalignant lesions and oral carcinoma occurs in the early stages [13, 14], which provides a survival rate above 70% at five years.

Some studies have linked early diagnosis of oral cavity cancer of the primary tumor location [15]. Thus, tumors of the lip, lingual or oral mucosa favors their diagnosis in an early stage, while their location on the oral floor or retromolar trigone is associated with late diagnosis [16].

Oral tumoral lesions can affect individuals of all ages. In our study, we showed that malignant lesions of

the oral cavity were found from the age of one year to 87 years. Most tumor lesions occurred in patients aged over 50 years, about 78.32% of cases, which makes us say that with age increases the risk of developing cancer of the oral cavity by aggregating harmful effects of carcinogens. Currently all researchers agree with the idea that chronic exposure to carcinogens can lead to genetic abnormalities in cells of oral mucosa, characterized by activation of proto-oncogenes and inactivation of tumor suppressor genes [17]. Under these conditions, cells have a marked tendency to accumulate additional genetic abnormalities due to genomic instability, leading to the emergence of phenotypically altered cells with rapid cell cycles, with decreased ability to repair replication defects and inefficient signaling of cellular apoptosis. The accumulation rate of acquired genetic abnormalities increases logarithmically with time [18]. Finally, these abnormal cells acquire malignant phenotype and lose their normal ability to differentiate, invade the basement membrane, proliferate in the connective tissue having a destructive local effect and metastasize through blood and lymph vessels.

Nowadays, from the histopathological point of view it is considered that oral neoplasms develop gradually, following a series of steps, from simple dysplasia, to the moderate and severe one, and then to "*in situ*" carcinoma and finally to invasive disease. To improve prognosis in oral cancer, early detection is necessary for those premalignant oral lesions that may evolve into invasive tumors [19–21].

Regarding histopathological type of oral tumors, in our study, the incidence of squamous cell carcinoma was 92%. Among them, well-differentiated squamous cell carcinoma was found in 53.05%, moderately differentiated squamous cell carcinoma was found in 31.30% and poorly differentiated squamous cell carcinoma in 15.65% of studied patients. Our data are similar to other studies that have shown that oral cavity squamous cell carcinoma is the most important and frequent malignant neoplasm accounting for over 90% of all malignant neoplasms [22].

Oral cavity cancer etiology is multifactorial and the most important risk factors are smoking and alcohol consumption. Ryerson AB *et al.* [23] have reported that the risk factors most frequently cited for oral cavity and pharynx cancer was smoking tobacco and drinking alcohol. Tobacco contains over 30 known carcinogens, most of them being polycyclic aromatic hydrocarbons and nitrosamines. According to some authors [24], smoking cessation decreases the risk of oral dysplasia, reaching values similar to those who never smoked after a lapse of 15 years sobriety.

Regarding the tumorigenic action of alcohol, a large number of epidemiological studies have shown a correlation between alcohol intake and cancer [25]. Schütze M *et al.* [26] showed how in Western Europe (Denmark, France, Germany, Greece, Italy, Netherlands, Spain, UK), a significant number of cases of cancer can be assigned to alcohol consumption.

The mechanisms by which alcohol consumption exerts its carcinogenic effect have not been fully defined, although plausible events include: a genotoxic effect of acetaldehyde, nutritional deficiencies, changes in the methylation process, collapse of the immune system, etc. [27, 28]. Alcohol may be an important factor in initiating malignancy, either by increasing the expression of certain oncogenes or by reducing the ability of cells to repair DNA, thus increasing the likelihood of oncogenic mutations. DNA methylation is an important regulator of gene expression. Reduction of tumor promoter gene methylation has been proposed as a possible mechanism for the development of cancer [25].

Molecular and cellular biology techniques have brought new data for understanding the carcinogenesis process. Numerous recent studies have focused on the tumor suppressor gene TP53, analyzing its gene and protein status. TP53 gene mutation is the most frequent genetic alteration found in human tumors [29]. TP53 mutations can result in over-production of inactive p53 proteins, which accumulate in the epithelium. In our study, p53 protein appeared intensely positive in 86% of oral squamous cell carcinomas, which suggests that these tumors develop due to the accumulation of genetic errors in the epithelium covering the oral mucosa.

Ki-67 proliferation factor study showed that in poorly differentiated carcinomas immunohistochemical reaction occurred more intensely positive than well-differentiated carcinomas, indicating an acceleration of mitotic rhythm and more aggressiveness in poorly differentiated carcinomas.

Other authors [31, 32] obtained similar results. They have shown that in oral squamous carcinomas, the analysis of Ki-67 immunoexpression indicated positivity in all investigated cases, but the percentage of immunostain index was different, correlated with the degree of neoplastic differentiation.

Conclusions

In our study, out of 143 oral cavity tumors, 125 were malignant tumors, of which 115 (92%) were squamous cell carcinoma. Of squamous cell carcinomas, 85 (73.91%) cases were diagnosed as stage III and IV tumors. Most of the tumors were located on the lower lip (36.4%), lower jaw (25.2%), floor of mouth (12.6%) and the tongue (8.4%). In relation to the social environment, 91 (64%) patients were from rural areas and only 52 (36%) patients in urban areas. Compared to the sex of patients, out of 143 patients with tumoral lesions of the oral cavity, 96 (69%) were males and 45 (31%) patients were females. Most tumor lesions of the oral cavity have been diagnosed in people over the age of 50 years. Most of the patients were over 50-year-old (124 patients, 86.71%), with a peak in the 61–70 years decade.

Contribution Note

All authors have equally contributed to the manuscript.

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