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Clinical-Pathological Analysis of 37 Oral Squamous Cell Carcinomas in Tucumán, Northwestern Province of Argentina: Immunohistochemical Study of p53 In Selected Cases

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Oral squamous cell carcinoma; Argentina; Tucuman; risk factors; Incidence, histological grade, p53

1. Abstract

Oral squamous cell carcinoma (OSCC) represents 3% of all malignant neoplasms. Considering the observed regional occurrence of OSCC, the aim of this study was to analyze the incidence and clinical characteristics of patients with primary carcinoma of the oral cavity diagnosed in the province of Tucumán, in the Northwestern of Argentina, where habits and lifestyle can play a central role in its incidence. A cross-sectional, descriptive and exploratory study was conducted with 37 clinical records of patients with OSCC. The study included the analysis of clinical and histological parameters, risk factors and the presence of p53 mutations in relation to the histopathological grade and the carcinoma in situ (CIS) component. OSCC is the most frequent oral malignancy in Tucumán. Most of the patients (81.1%) were male, and the mean age range was 65.5 years. The analysis of risk factors showed that 89.2% of the patients smoked. Most of the tumors (38%) were located on the tongue and the greater histological aggressiveness of OSCC was observed in this location. Overexpression of p53 was observed in OSCC with CIS component. OSCC is the most frequent oral malignancy in Tucumán. The data also agree with reports from other locations in terms of sex, age, location, and greater histological aggressiveness of OSCC in the tongue. The overexpression of p53 in OSCC with a CIS component could indicate the occurrence of its mutation in early lesions. The mutation in p53 in non-invasive lesions found in the adjacent surface epithelium could have predictive potential for progression or recurrences from inadequate surgical margins.

2. Introduction

Cancers of the lip and oral cavity occupied the 18th position in the list of new cases and deaths among 36 cancers in 185 countries according to GLOBOCAN 2020 [1]. Oral squamous cell carcinoma (OSCC) is the most frequent malignancy of epithelium origin affecting the oral cavity [2, 3]. It is highly frequent in certain regions of the world, such as in South Central Asia (eg, India, Sri Lanka, and Pakistan) as well as Melanesia (Papua New Guinea); and it is associated with a high mortality rate, being the leading cause of cancer death among men in India and Sri Lanka [1, 4]. OSCC is considered an elderly disease because it is seen predominantly in patients over 65 years; however there is a general increase in the incidence of tongue OSCC worldwide, especially in younger patients [5]. There is also a difference between genders, being OSCC predominant in males; however, the male/female differential is decreasing, maybe associated with changes in tobacco and alcohol habits in women [6]. It is also more common in lower socioeconomic groups and in ethnic minority groups. In addition, the decreased survival observed in these populations has been associated with a higher prevalence of presenting at later stages and a lower prevalence of being insured [7]. A wide range of genetic, environ-

mental, and behavioral factors contribute to the risk of oral cancer. Risks are dominated by tobacco, both smoked and smokeless, and heavy alcohol consumption [8].

Despite improved surgical treatments and new therapies have not led to significant survival; early diagnosis is a key factor in improving oral cancer control and reducing morbidity and mortality [9].

OSCC shows a high incidence of alterations in the tumor suppressor gene p53. This gene is involved in pro-apoptotic activities; it mutates during the development of many human malignancies; and in this sense, pP53 mutation and overexpression are closely related events. An overexpression of the p53 in oral cancer has been associated with poor prognosis; the analysis of these alterations provides important information on the diagnosis, prognosis and therapy of affected patients, being an indicator of using more aggressive adjuvant therapies in patients with high-risk [10, 11]. Every year 3,000 new cases of oral cancer are detected in Argentina. In the same period, it causes the death of between 800 and 1,000 people (approximately two per day) [12]. As in the rest of the world, habits and lifestyle play a central role in its incidence. Tobacco, betel quid with tobacco, betel quid without tobacco, smokeless, alcohol and the human papillomavirus (HPV) are the main causes (agents classified as carcinogenic to humans by the International Agency for Research on Cancer in 2022) and other potencial etiologic factors have also been reported. The consumption of hot drinks, especially the drinking of mate [13, 14], the coca chewers in the Northern region [15], the high red meat intake are also inflammatory potential factors related to OSCC [16], added to the chronic mechanical irritation that may be considered a risk factor or can also influence other important risk factors for OSCC [17]. A representative study of the Argentine population carried out at the University of Buenos Aires showed a series of 274 patients with oral carcinomas between the years 1992-2000. The survival rate of the studied population was 39% at 60 months, being the locations with the worst prognosis the floor of the mouth and the tongue [18]. This poor prognosis was mainly due to the large number of cases of oral cancer that were diagnosed in advanced stages.

Considering the observed regional incidence of OSCC in different countries of the world, this study aimed to analyze the incidence and clinical characteristics from patients with primary carcinoma of the oral cavity diagnosed in the province of Tucumán, in the Northwestern of Argentina, analyzing clinical and histological parameters, risk factors and the presence of p53 mutations.

3. Material and Methods

3.1. Study Design

The study was performed with tissue biopsies obtained of the General Pathology Service of the Hospital Angel C. Padilla (San Miguel de Tucumán, Tucumán, Argentina), a reference public hospital in the province, between 2002 and 2010. A cross-sectional, descriptive and exploratory study was conducted with 37 clinical records of patients with OSCC. The study included histopathological analysis and the evaluation of the protein p53 in tumor samples.

3.2. General and Medical Variables Studied

General and medical records of individuals included gender, age, tumor location and risk factors. The variable gender was registered as male and female, and the age in years at the time of diagnosis. The risk factors evaluated were: smoking (yes-no), and alcoholism (yes-no).

Tumor location (lip, gingiva, tongue, jugal mucosa, soft palate, hard palate, tonsil abutment, floor of the mouth, upper alveolar ridge, lower alveolar ridge, and oropharynx) was also analyzed.

3.3. Histopathological Analysis

Tumour histological sections processed routinely by paraffin inclusion were stained with Hematoxylin & Eosin and analyzed under microscopy. Histological grade I, II, or III was established based on the degree of differentiation (well differentiated, moderately differentiated and poorly differentiated, respectively). Keratinization was described as mild, low, moderate, abundant or absent; cellular and nuclear pleomorphism as mild, moderate or marked; and mitotic index was analyzed in 10 random fields at 400x of magnification. The adjacent mucosa was studied for the presence of carcinoma in situ (CIS), dysplasia and hyperplasia. Surgical margins (positive/negative) and perivascular, perineural and adjacent tissue invasion were also evaluated.

3.4. Immunostaining Technique for p53

Twelve representative cases of studied OSCC were selected, to carry out p53 immunostaining techniques. Briefly, the tissue sections were mounted on silanized slides, after deparaffinization and hydration, antigenic recovery was carried out in citric acid buffer pH 6, followed by blocking the endogenous peroxidase with 3% H2O2 in methanol, and incubated overnight at 4° C in a humidity chamber with monoclonal p53-D07 antibody (Novocastra, Leica Biosystem, Newcastle, UK) labeled with peroxidase. Sections were then treated with ABC solution (Vectastain ABC Elite kit, Vector Labs, Burlingame, USA), and incubated with DAB (diaminobenzidine) substrate (Vector Labs, Burlingame, USA). Counterstaining was carried out with hematoxylin, and finally the samples were dehydrated and mounted. p53 immunostaining was assessed using a double evaluating system calculated by the intensity of staining (0, negative; 1, weak; 2, moderate; 3, strong) with the percentage of stained neoplastic cells in a low-power field (1 % to 10%; 2, 11% to 50%; 3, 51% to 75%; 4, >75%). The immunohistochemical staining pattern (focal vs diffuse) was also recorded.

3.5. Statistical Analysis

The study design was cross-sectional with an exploratory-descriptive statistical treatment. The statistical software SPSS Statistics 17.0 was used and a 5% significance level was set as acceptable.

4. Results

From 2002 to 2010, a total of 264 biopsies of the buccal mucosa were performed in the General Pathology Service of Hospital Ángel C. Padilla, of which 52 cases (19.70%) corresponded to malignant neoplasia (Table 1).

Malignant neoplasms (52 cases) presented the following distribution: Oral Squamous Cell Carcinoma (OSCC), 39 cases (75%); Adenocarcinoma, 2 cases (3.85%); Adenoid Cystic Carcinoma, 6 (11.54%); Non-Hodking Lymphoma, 1 case (1.92%); Melanoma, 1 case (1.92%); Kapossi sarcoma, 2 cases (3.85%) and Metastasis, 1 case (1.92%). For the study presented in this work, 37 of the 39 cases with a diagnosis of OSCC originating in the buccal mucosa were selected, since they had complete data.

Table 1: Types of oral cavity pathologies of a total of 264 cases; Cases are described as the number (n) and the percentage (%) from the total

Pathology	Cases (n)	Cases (%)
Malignant neoplasm	52	19.7
Dyisplasia	2	0.8
Stomatitis	41	15.5
Glossitis	7	2.6
Pseudotumor lesions	45	17.1
Bening neoplasm	32	12.1
Gingival pathology	21	7.9
Minor salivary gland pathology	48	18.2
infectious pathology	8	3
Immunopathology	8	3
Total	264	100

5. Univariate Study

5.1. Clinical Variables

The demographic characteristics of the study group population showed that of the 37 cases, 30 (81.1%) were male, and 7 (18.9%) were female (Table 2). The age range of the patients was from 48 to 81 years, with a mean of 65.5 years. Two risk factors were analyzed: smoking and alcohol consumption. The results showed that 33 patients (89.2%) smoked and only 6 patients (16.2%) consumed alcohol (Table 2). Tumor location analysis was performed in 34 of the 37 because the remaining 3 did not record the exact location in the clinical history, although they corresponded to intraoral location. Most of the tumors analyzed (38%) were located on the tongue, while the rest corresponded to the buccal mucosa, soft palate, gingiva, floor of the mouth, lip, oropharynx, hard palate, tonsillar pillar, upper and lower alveolar ridge (Table 2).

Table 2: Analysis of general and clinical features associated to OSCC; The number in parentheses next to each evaluated parameter indicates the total number of cases analyzed. Cases are described as the number (n) and the percentage (%) from the total.

Analysed parameter	Cases (n)	Cases (%)
Distribution by gender (37)		
Male	30	81.1
Female	7	18.9
Risk factor (37)		
Smoking (yes)	33	89.2
Alcoholism (yes)	6	16.2
Tumor location (34)		
Tongue	13	38.2
Jugal mucosa	5	14.7
Soft palate	5	14.7
Gingiva	3	8.8
Floor of the mouth	2	5.9
Lip	1	2.9
Oropharynx	1	2.9
Hard palate	1	2.9
Tonsillar pillar	1	2.9
Upper alveolar ridge	1	2.9
Lower alveolar ridge	1	2.9

5.2. Histopathological Evaluation

The degree of differentiation of OSCC was analyzed in 32 of the 37 samples. OSCC Grade II was observed in 17 cases (53.1%); OSCC Grade III in 13 cases (40.6%) and was not evaluable in 2 cases (6.3%) (Table 3). Representative photos from biopsies of cases with these degrees of differentiation are shown (Figure 1).

The parameters for the determination of the histological grade were: the degree of keratinization, the cellular pleomorphism, and the mitotic index were analyzed. Degree of keratinization was evaluated in 31 samples and showed moderate keratinization in 52% of the cases, while the rest (48%) presented scarce keratinization (Table 3). Cellular pleomorphism was a characterization found in most of the samples, being marked in more than half of the evaluated cases (18 of 31) and moderate in 11 cases (35.5%) (Table 3). Mitosis counting was performed in 30 of the 37 samples. Results between 0 and 5 were obtained, 0 being not evaluable. More than 50% of the samples presented 3 or 4 mitosis (Table 3). The observation of the adjacent mucosa showed that of 32 samples analyzed, carcinoma in situ (CIS) was observed in 12 cases (37.5%) (Figure 2). There was a higher prevalence of epithelial dysplasia, epithelial hyperplasia and papillomatosis, which were observed in more than 70% of the samples analyzed (71.4%, 86.7% and 73.3% of the cases, respectively) (Table 3). Positive surgical margins were observed in 15 of 17 cases (88.2%). The perivascular and submucosa invasion were those that predominated (93% and 97%, respectively) in the analyzed samples; while the invasion to perineural tissue, muscle, salivary glands or bone tissue presented percentages of 30%, 36%, 24% and 4%, respectively (Table 3).

Table 3: Histopathological evaluation of OSCC; The number in parentheses next to each evaluated parameter indicates the total number of samples analyzed. Cases are described as the number (n) and the percentage (%) from the total.

Analysed parameter	Cases (n)	Cases (%)
Degree of differentiation (32)		
Not evaluable	2	6.2
Grade II	17	53.1
Grade III	13	40.6
Degree of keratinization (31)		
Mild	0	0
Scarce	15	48.4
Moderate	16	51.6
Cellular pleomorfism (31)		
Not evaluable	1	3.2
Mild	1	1.2
Moderate	11	35.5
Marked	18	58.1
Mitosis counts (30)		
0	7	23.3
1	0	0
2	3	10
3	11	36.7
4	7	23.3
5	2	6.7

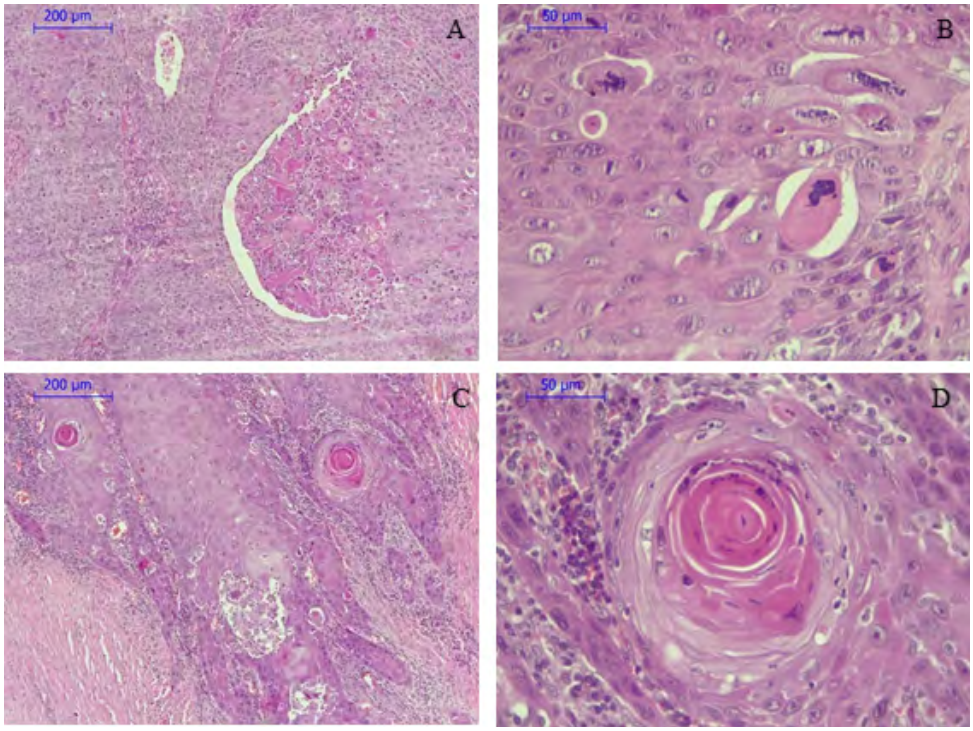

Figure 1: Representative photos from biopsies of OSCC with different degrees of differentiation. A, Marked pleomorphism and areas of necrosis. B, Atypical mitoses, pleomorphism in HG OSCC. C and D, Moderate pleomorphism and horny pearls in moderately differentiated OSCC.

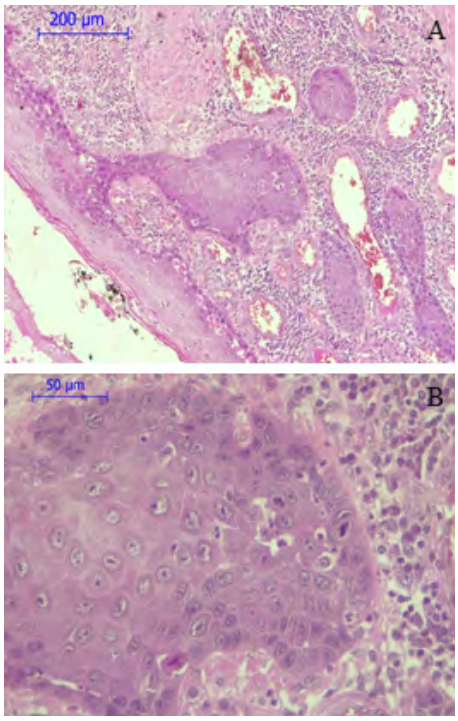

Figure 2: Representative photos from biopsies of OSCC with the presence of carcinoma in situ (CIS). A, CIS in the adjacent surface epithelium, H&E, 100x. B, CIS, droplet ridges, H&E, 400x.

5.3. Bivariate Study

The analysis of the associations between location and degree of differentiation showed that 83% of the tongue OSCC were Grade III. No associations were found with the risk factors investigated (smoking and alcohol consumption). It was detected an association ($p < 0.05$) between degree of differentiation and keratinization or pleomorphism (Table 4). The 88% of OSCC Grade II had moderate keratinization and 100% of OSCC Grade III had marked pleomorphism.

Table 4: Histological characteristic of adjacent mucosa and tissue invasion; The number in parentheses indicates the total number of samples analyzed. Cases are described as the number (n) from the total evaluated.

Histological observation	Cases (n)
Adjacent mucosa	
Carcinoma in situ (32)	12
Epithelial dysplasia (28)	20
Epithelial hiperplasia (30)	26
Papillomatosis (30)	22
Tissue invasion	
Perivascular (29)	27
Perineural (30)	10
Submucosa (33)	32
Muscular (33)	12
Salivary glands (33)	8
Bone tissue (28)	1

5.4. Immunostaining for p53

Fifty percent (5/10) of the samples analysed (one case was excluded) presented positivity greater than 45% for p53 immunostaining. Of them, 40% had an associated CIS component. The (Table 5) shows the immunostaining results for p53 for the OSCC cases studied, and (Figure 3) shows representative photographs

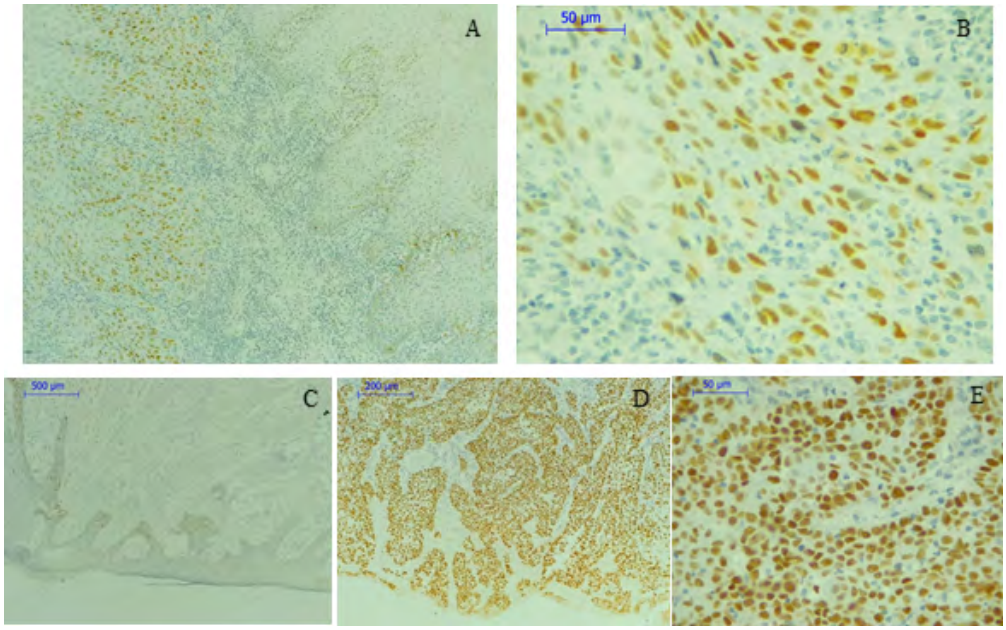

Figure 3: Representative photos of p53 immunostaining. A, Areas of epithelial dysplasia in surface epithelium. B, Invasive areas of OSCC with p53 expression of 45%. C, Carcinoma in Situ (CIS) in superficial epithelium (magnification 100x). D and E, the same CIS, ulcerate zone with >75% p53 reactivity

Table 5: Associations detected with degree of differentiation of OSCC; Data show the number of cases (n) from a total of 30 for each; ‡Association degree of differentiation / keratinization: chi-square ($\chi 2$) = 23.35; $p<0.001$; †Association degree of differentiation / pleomorphism: chi-square ($\chi 2$) = 48.31; $p<0.001$

Degree of differentiation	Keratinization‡		Cellular pleomorphism†		
	Scarce	Moderate	Mild	Moderate	Marked
Not evaluable	1	1	0	0	0
Grade II	2	15	1	11	5
Grade III	11	0	0	0	13

Table 6: Immunostaining for p53 in twelve representative cases of OSCC; ‡p53 was determined by immunohistochemistry and the results expressed as negative or positive (with the percentage of positive cells)

Case	Diagnosis	Degree of differentiation	P53‡
Case N°1	OSCC	II	Positive (90%)
Case N°2	OSCC		Negative
Case N°3	OSCC	III	Doubtful
Case N°4	OSCC	III	Negative
Case N°5	OSCC	II	Negative
Case N°6	OSCC	II	Negative
Case N°7	OSCC	II	Negative
Case N°8	OSCC	II	Positive (without %)
Case N°9	OSCC	II	Positive (95%)
Case N°10	OSCC	II	Positive (45%)
Case N°11	OSCC	III	Positive (95%)
Case N°12	OSCC	III	Positive (90%)

5.5. Bivariate Study

No associations were detected between the variables ($p > 0.05$). However, of the cases with positive immunostaining for p53, 40% (2/5) had carcinoma in situ; 60% (3/5) had positive surgical margins; 80% (4/5) presented moderate keratinization.

6. Discussion

The study of OSCC is of great importance due to its high morbidity and mortality. Most of the studies published in Argentina reported cases associated with patients from Buenos Aires (the capital) or Córdoba (one of the biggest provinces) [18, 19), and as explained in the aim of this work, it is important to consider the great variability in the incidence of OSCC, even between regions of the same country. Thus, knowing the incidence and clinical characteristics of OSCC in Tucumán, province of the Argentine Northwest, with particular customs and lifestyles, provides data that can be useful for the application of health policies oriented to prevention, early diagnosis and better treatment of the OSCC in this region. The results showed agreement with the literature regarding gender, age, location and greater histological aggressiveness of the OSCC in the tongue. Our study revealed a higher incidence of OSCC in men, with a male/female ratio of 4.3: 1. This is in agreement with the GLOBOCAN 2020 oral cancer report [1]). However, the ratio is higher than the reported average for Argentina (2.3) [20], and show a significant difference with the study by Brandizzi et al. (2008) in which the authors presented a series of cases from Buenos Aires with a male/female ratio of 1.24 / 1 and argued that due to changes in lifestyle, mainly the increase in the number of women smokers in recent decades, there is a worrying increase in the incidence of oral cancer in women in the city of Buenos Aires [18]. Regarding the age distribution, our series of cases consisted of individuals older than 48 years old with a mean of 65.5 years. These data agree with the literature and those obtained from other regions of Argentina [18, 21]; however, unlike other reports, we did not observe an increase in incidence in young adults, under 40 years of age [22]. In concordance with other reports, the tongue was the most frequent localization of oral cancer in our series (38.2%). The study in Buenos Aires [Argentina) reported a prevalence of 30% [18]. A similar prevalence was also described in regions from other countries, such as 37% in the Southeastern of Brazil [23], and 35.3% in Madrid (Spain) [6].

The evaluation of p53 protein expression in the biopsies was carried out because of the loss of stability of tumor suppressor genes, such as p53, causes the inability to respond to the control mechanisms that regulate cell division; which sometimes leads to the development of neoplasms and their evolution towards more aggressive tumor processes [24]. The p53 gene is one of the most representative suppressor genes; it is mutated in approximately half of almost all types of cancers that originate in a wide spectrum of tissues [25]. It was reported that high percentages of OSCC cases and premalignant oral dysplasia lesions were positive for p53 [26]. In our case series, overexpression of p53 in OSCC with a CIS component could indicate the appearance of its mutation in early lesions, supporting the idea that the mutation in p53 is an early event in oral carcinogenesis [27, 28]. It was also described that expression of p53 in histologically negative surgical margins can be used to predict the local recurrence of OSCC [29].

Knowledge of the genetic alterations that favor the onset of cancer can facilitate the early diagnosis of primary tumors and possible recurrences, which can determine the success of treatment. A systematic review reported that the frequency of oral leukoplakia is low in young patients; from the study of young patients with oral leukoplakia as the only oral epithelial dysplasia was found in 34.7% of the patients, of which 6.9% presented malignant transformation [30]. In another systematic review, significant risk factors for malignant transformation of oral leukoplakia are advanced age (over 50 years), female gender, OL involving the oral tongue,

non-homogeneous clinical type, and the presence of epithelial dysplasia [31]. Leukoplakia, erythroplakia and submucous fibrosis are highly associated with the development of oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC) [32]. A three-tiered the OED grading system is maintained in the last 5th edition of the World Health Organization's classification of Head and Neck Tumors [33]. The key is an early diagnosis of Oral Potentially Malignant Disorders (OPMD) or initial stages of cancer that implies the performance of mini-invasive surgeries, and a better prognosis and quality of life for the patient. In this sense, it was reported that moderate/severe dysplasia bears a much higher risk of cancer evolution than mild dysplasia [34]. The present study has limitations, such as the low number of cases and the failure to include other risk factors in the patient's medical records. It is also important to highlight that the data obtained allowed us to verify a scarcity of data in the clinical records of references, from a general hospital, regarding the clinical appearance of the lesions, symptoms, exact location, etc. The incorporation of dental surgeons, stomatologists and oral pathologists in the teams of general hospitals can improve the medical records for these specific areas. To our knowledge, there is no similar publication with specific data from the province of Tucumán or from the Northwestern region of Argentina. Thus, the data presented will be useful to know the current situation of the OSCC in the province and contribute to applying policies for the early diagnosis of OPMD and oral cancer, and the control of risk factors, in addition to promoting adherence to campaigns of prevention of both national and Latin American to fight against oral cancer.

7. Conclusion

Results showed that OSSC, similar to data obtained from other Argentinian regions and other countries, is the most frequent oral malignant neoplasm in Tucuman. Data also agree with the literature regarding gender, age, location and greater histological aggressiveness of the OSCC in the tongue. However, in our population the increase in female and younger patients (below 40 years old) that is being reported in other regions was not observed. The overexpression of p53 in OSCC with a carcinoma in situ (CIS) component indicates that the occurrence of its mutation in non-invasive lesions found in the adjacent surface epithelium could have predictive potential for progression and has the potential to be used to predict progression or recurrences from inadequate surgical margins.

8. Conflict of Interest

The authors declare no competing interests

9. Funding

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10. Ethics

All procedures performed in the present study were in accordance with the ethical standards of the institutional research committee. The present study has the approval of the ethical committee (Comisión de Bioética of Facultad de Odontología, Universidad Nacional de Tucumán) with the number 018-2022.

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